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Abstracts

VOLUME II

Part 2

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Neurocytology

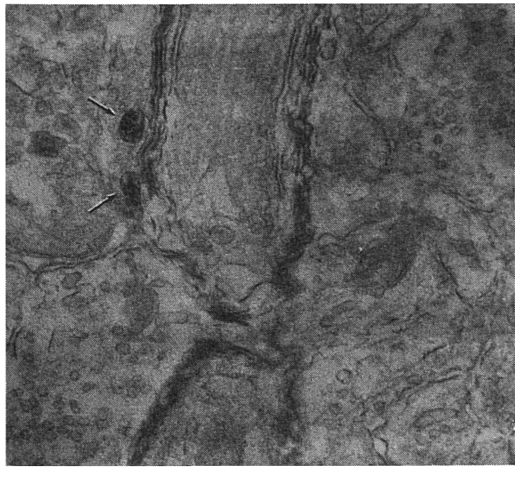
899 MICROPEROXISOMES IN THE CENTRAL NERVOUS SYSTEM OF THE POSTNATAL RAT. Gail Arnold and Eric Holtzman. Dept. of Biological Sciences, Columbia University, New York, N.Y. 10027.

Previous work in this laboratory using the alkaline-DAB cytochemical method for catalase has demonstrated the presence of microperoxisomes in both the peripheral and central nervous systems of the adult rat. Cytochemically reactive bodies are found in neurons of sympathetic ganglia and also in neurons of catecholaminergic regions of the brain. Neurons of dorsal root ganglia and of cerebral and cerebellar cortex contain relatively few reactive structures. Schwann cells and most glial cells contain reactive microperoxisomes, generally inhigher concentrations than are found in neurons.

The present study is concerned with the distribution of catalase-containing microperoxisomes during the postnatal period, when maturation of the cortical regions, and myelination of the entire brain, are occurring. The most evident features we have observed thus far are 1) In the early postnatal period, many cell profiles in thin sections of the cerebral cortex contain appreciable numbers of reactive bodies. The cells are relatively undifferentiated at this stage. 2) In several regions of the brain, examined during the formation of myelin, cytochemically reactive microperoxisomes frequently are observed in cytoplasmic processes immediately adjacent to myelin sheaths. 3) In the locus coeruleus, all postnatal stages examined show moderate numbers of reactive microperoxisomes in neurons and also in glia. An interesting difference between adult and 18-day postnatal neurons was the frequent occurrence of microperoxisomes in presynaptic terminals in this region of the immature brain. 4) The maturing choroid plexus of the IV ventricle contains modest numbers of reactive microperoxisomes.

Little is known of peroxisome roles in animal cells in general. However, there is some evidence that peroxisomes in several cell types are involved in lipid metabolism, and in line with this, our results may indicate participation of such organelles in some aspect of the elaboration of myelin.

Supported by NIH Grant No. NS 09475.



The arrows indicate catalase-reactive bodies in a cytoplasmic process adjacent to a forming myelin sheath in the locus coeruleus from an 18-day postnatal rat.

900 E.M. IDENTIFICATION OF NON-PRIMARY AFFERENT TERMINALS IN THE GRACILE N. WITH SOME OBSERVATIONS ON THE APPEARANCE OF "GROWTH CONES" IN CHRONICALLY DEAFFERENTED ADULT CATS. L. Ellis * and A. Rustioni, Depts. of Anat. and Physiol., University of North Carolina, Chapel Hill, N.C. 27514.

Afferent fibers to the dorsal column nuclei originating from spinal grey matter and ascending in the dorsal and lateral funiculi have been demonstrated in cats with light microscopical techniques (Rustioni, 1973, 1974). In order to identify synaptic endings of these non-primary ascending fibers, transection of the dorsal and lateral funiculi were made at upper lumbar levels in adult cats one year after bilateral lumbo-sacro-coccygeal dorsal rhizotomy. By this approach, sufficient time for removal of degenerated dorsal root terminals is allowed and selective interruption of non-primary afferents to the gracile n. is possible. Two to five days after spinal transection, all animals were perfused with aldehydes (1% paraformaldehyde and 1% glutaraldehyde in 0.12M phosphate buffer, pH7.2), postfixed with 2% OsO_4, dehydrated in graded alcohols, and embedded in Spurr's low viscosity medium. The degeneration and removal of synaptic endings identifiable as non-primary afferent terminals appears to have a sudden onset and to proceed rapidly. Boutons at various stages of degeneration, as well as engulfed by glia, are present after three days survival, but are almost absent in the case so far examined with shorter survival time. At variance with dorsal root terminals, non-primary afferent endings do not appear to establish axo-axonic contacts, are presynaptic to small dendritic profiles and undergo degenerative changes of the "dark" as well as of the "light" type. For these characteristics, non-primary afferents to the gracile n. are similar to terminals of cortical origin in the same nucleus (Rustioni and Sotelo, 1974). This similarity is especially interesting since these two afferent systems overlap in their distribution within the gracile n. and both spare the "clusters" region.

Several abnormal features are encountered in the gracile n. of these chronically deafferented animals. Denervated postsynaptic specializations, probably left vacant by removal of dorsal root terminals, are commonly found opposite glial or dendritic profiles. Dendrites may contain a membranous sac filled with "vesicles" of variable size and shape, usually larger than synaptic vesicles. In some instances, the "vesicles" contain an eccentrically located, small (25-40nm), electron dense granule. Postsynaptic elements with numerous, irregularly shaped cisterns, large (80-150nm) dense core vesicles, and ribosomes are identified as dendritic "growth cones" on the basis of their similarity with comparable structures described in the developing nervous system. Abnormal presynaptic profiles containing many irregular cisterns and dense core vesicles are also found. On the basis of the material so far examined, identification of these structures as axonal "growth cones" is still uncertain.

Control material is currently under study to determine whether the appearance of "growth cones" is a consequence of the chronic deafferentation, the spinal transection, or a combination of the two procedures. These results represent a further indication of plastic properties in the CNS and suggest a potential mechanism by which new connections may be established following denervation.

Supported by NIH Grant NS12440 and by the A.P. Sloan Foundation.

901 THE EFFECT OF $(But)_2$cAMP ON IN VIVO NERVE REGENERATION. M. R. Gershenbaum* and F. J. Roisen* (SPON: S. Rosner), Department of Anatomy, CMDNJ-Rutgers Medical School, Piscataway, New Jersey 08854

The ability of N^6, 0^2-dibutyryl adenosine 3', 5'-monophosphate [$(But)_2$cAMP] to stimulate in vitro nerve development (1) has prompted several studies on the effects of the nucleotide on in vivo nerve regeneration. Conflicting reports (2-4) have been published. This work was undertaken to further clarify the role of $(But)_2$cAMP in the regeneration of neuronal tissue.

The left sciatic nerves of male Holtzman rats (200-240gm) were exposed and crushed with a hemostat for 3 seconds. The serrated jaws of the hemostat were taped to limit damage. Surgical thread was positioned proximal to the crush to facilitate identification of this region during morphological studies. After a 24 hour recovery period, animals received daily intramuscular injections of either $(But)_2$cAMP (50 mg/kg) or saline (0.9%) adjacent to the lesion. The rats were coded to minimize experimentor bias and tested daily, prior to injection, for return of sensorimotor function (SMF). An estimate of SMF was obtained by placing the hind limb over the aperture of a shutter-controlled, high intensity light box. The time required for limb withdrawal was recorded to the nearest second. If no response occurred the trial period was terminated after 30 seconds.

$(But)_2$cAMP and saline-treated rats showed no difference in the rate of return of SMF in the first 10 days following the crush. The nucleotide-treated animals exhibited a significant decrease in response time by day 12 and were completely recovered by day 18. Saline-treated rats did not show full recovery until day 26. (See Figure 1.)

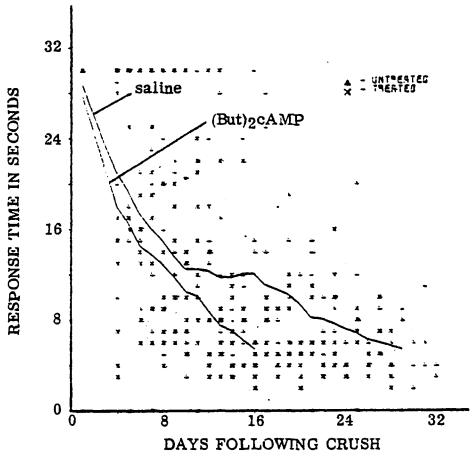


Figure 1

Preliminary ultrastructural examinations of comparable regions of sciatic nerves demonstrated increased numbers of myelinated fibers in nucleotide-treated animals when compared with saline-treated controls. Scanning electron microscopy was employed to correlate the ultrastructural observations with changes in surface morphology.

The raw data for this experiment was key punched and fed to an IBM 370-168 computer coupled with a Calcomp plotter. This data was examined by analysis of variance, regression coefficients and statistics for fit of dependent variables. The curves were drawn according to the first degree polynomial smoothing of five points.

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Supported by USPHS grant NS11299.

902

WITHDRAWN BY AUTHOR

903 THE DEVELOPMENT OF MAST CELLS AND NEUROLIPOMASTOCYTES IN THE BRAINS OF RATS. Mohamed Z. M. Ibrahim. Dept. Anat., Faculty Med., Univ. Iowa, Iowa City, IA. 52242.

Ibrahim (J. neurol. Sci. 21:431, 1974) has drawn attention to the fact that certain granular cells found in the leptomeninges of mammalian CNS, and within the outermost parts of basement membrane systems of blood vessels inside it, are closely allied to mast cells (MCs). These cells, often referred to erroneously as "pericytes", or as "perivascular cells", "adventitial cells" or "perithelial cells", etc., he named "Type II MCs" or "neurolipomastocytes (NLMs)", (Ibrahim et al., Anat. Rec. 184:434, 1976 and in preparation); this more accurately describes their most dominant features. In the case of the rat, both these NLMs, as well as more orthodox MCs, follow roughly the same sequence of first appearance in the leptomeninges and then later inside brain substance (Ibrahim, 1968, 1974; Krüger, Experientia 30:810, 1966). The NLMs thereafter become ubiquitous while the MCs become more or less confined to the dorsal thalamic regions (Ibrahim, 1974). How the shift in distribution of both cell types occurs, what the ultrastructural morphological changes that accompany the maturation of the cells, and how these compare with known sequential alterations in MCs outside the CNS, are not known. Unknown as well are the developmental changes and interrelationship, if any, between the two types of cells.

In this presentation answers to some of these questions will be attempted through a study of developing Sprague-Dawley rat brains that have been examined by E.M. from about the 15th intrauterine to the 21st postnatal day of life. Thus, for instance, a nongranular precursor-type cell possibly for both cell forms probably exists. This has a far better developed Golgi complex and numerous pleomorphic mitochondria and profiles of smooth E.R., especially near the Golgi, than in either mature cell. Polyribosomes and fewer profiles of both smooth and rough E.R. also are seen but usually more peripherally. The nucleus, more irregular than in adults, is predominantly euchromatic. The two cell lines thereafter seem to develop apparently independently without obvious signs of one cell transforming into the other.

The MCs begin to acquire their granules which initially are not necessarily always adjacent to the Golgi but gradually come to occupy the entire circumference of the cell; all of the organelles then become more sparse. The granules also start to vary, as in the adult, in both density and texture and acquire characteristic halos and signs of partial degranulation (reticulation and confluence) with extrusion of intact or ghost-like granules.

The NLMs acquire their granules sometimes in close proximity to the Golgi and in a sequential manner with the smallest granules nearest that apparatus, as with the MCs. At other times dilated profiles of smooth E.R., apparently some distance from the Golgi, contain dense material and may "round off" into granular forms; the rest of the organelles then become less conspicuous. These dark granules do not acquire halos and are homogeneous, but some may then dilate, become irregular and lose density and substance appearing finely particulate. Many characteristic irregular vacuoles are seen early, as are long surface protoplasmic processes similar to those found at the surfaces of the MCs.

These sequential changes in both cell types appear to parallel each other and those already observed for MCs outside the CNS (Combs, J. Cell Biol. 31:563, 1966). The changes in the granule matrix of the MCs and the vacuolation of the NLMs probably reflect progressive changes in chemical composition (Combs, 1966; Csaba, Acta biol. Acad. Sci. hung. 20:205, 1969); the pallor and loss of substance of some granules in both cells, and the loss of others from the MCs, may reflect the discharge of the normal functions of these cells and not damage.

Further observations will be mentioned and discussed.

904 ANATOMY OF MOTOR NEURONS IN DROSOPHILA THORACIC GANGLION.
David G. King. Dept. of Biology, Yale Univ., New Haven CT 06520
A map of the thoracic ganglion of Drosophila, utilizing both skeletal and neural landmarks, was prepared from carefully oriented sets of serial sections prepared for light and electron microscopy. With this map the shape and location of neurons could be accurately described. Many neural processes were found to be identical from animal to animal. When traced through serial sections, such anatomically identifiable processes could be recognized unambiguously by the position of their profiles in relation to landmarks. Identified processes show consistent location of major branch points, destination of peripheral axon, and texture of cytoplasm. Motor neurons, which are among the largest neurons and which can also be studied physiologically (Harcombe and Wyman, in preparation), were most intensively examined for synaptic organization. Postsynaptic sites were found both on major processes and on smaller branches. Presynaptic sites were generally limited to smaller branches.

The giant fiber system in Drosophila was first studied by Power (J. Comp. Neur. 88:347-409, 1948) and by Levine and Tracey (J. Comp. Physiol. 87:213-235, 1973), both of whom describe a single pair of giant neurons extending from the brain to each of two different muscles (Fig. 1). This pair of giant fibers appears in the present study to consist of three distinct pairs of neurons (Fig. 2). On each side, a giant interneuron extends from the brain into the thoracic ganglion where it makes contact with two large motor neurons, one innervating the ipsilateral tergal depressor of the trochanter (TDT) and the other innervating the contralateral dorsal longitudinal muscle (DLM). In the region of this junction each of the three giant neurons receives extensive input from numerous small chemical synapses. (Supported by USPHS grants NS07314 and NS05198).

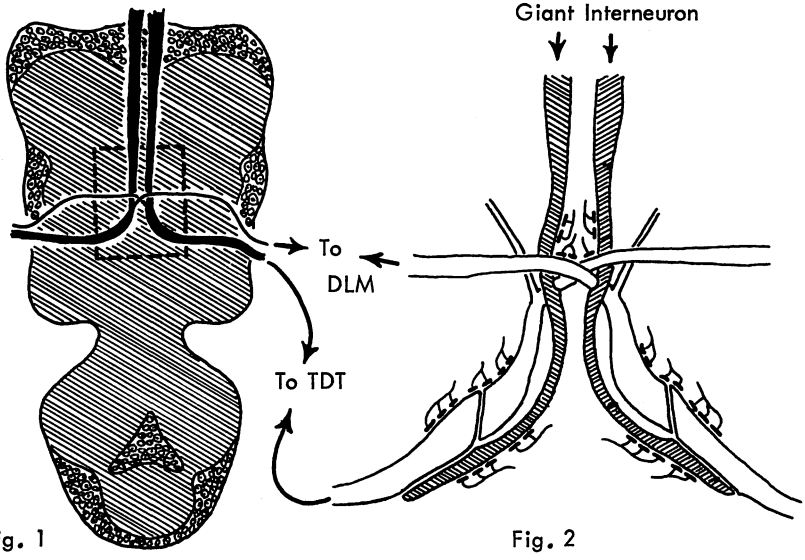
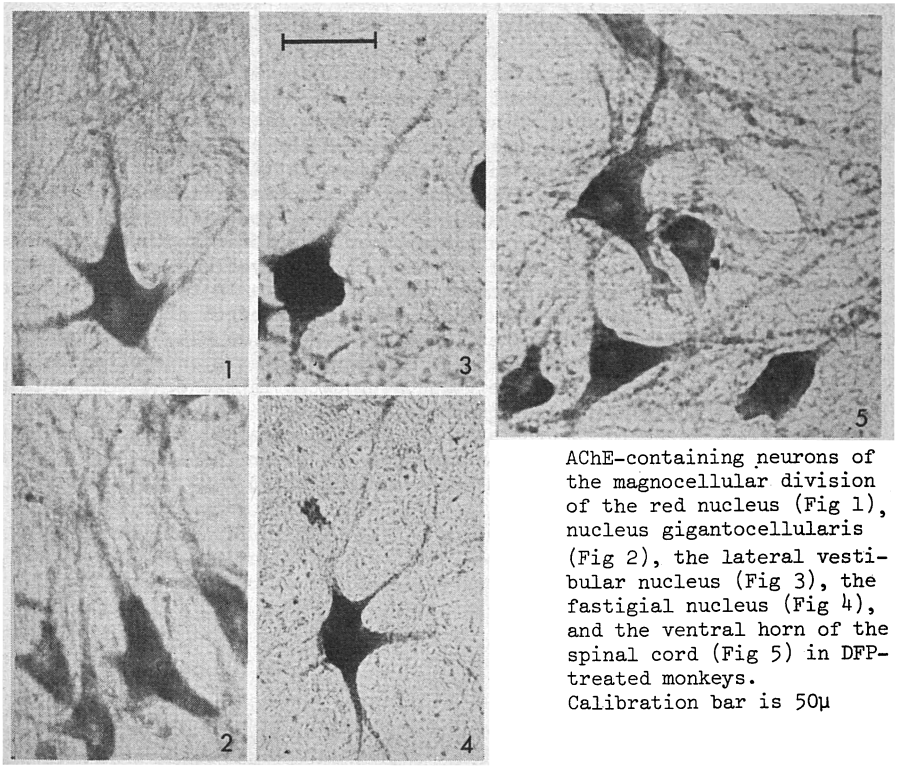


Fig. 1 Fig. 2

Fig. 1. Reconstruction of giant fibers projected onto a horizontal section through the thoracic ganglion (cf. Power, Fig. 7).
Fig. 2. Enlargement of outlined region in Fig. 1.

905 DISTRIBUTION OF ACETYLCHOLINESTERASE WITHIN VARIOUS GROUPS OF NEURONS OF THE MIDBRAIN, PONS-MEDULLA AND SPINAL CORD OF MONKEYS TREATED WITH DFP.
R. Marchand*, A. Parent and L.J. Poirier (SPON: P. Langelier) Neurobiology Lab. and Dept. Anat., Laval University, Quebec, QUE. G1K 7P4

Applying the pharmaco-histochemical technique based on the administration of di-isopropylfluorophosphate (DFP) of Butcher et al., (J. Neural Transm. 37: 127, 1975) we studied the distribution of acetylcholinesterase (AChE) within various groups of neurons of the brain stem and spinal cord in DFP-treated monkeys. Summarizing the observations an intense activity was disclosed in the soma and processes of the following groups of neurons: the motor nuclei of the III, IV, V, VI, VII, X and XII nerves including nuclei Edinger-Westphall, dorsalis of X and ambiguus, the neurons of the ventral horn of the spinal cord and nuclei intermedio-medialis and intermedio-lateralis; neurons of the dorsolateral nucleus of the raphae and neurons associated with the brachia conjunctiva at the level of their decussation; large neurons located in the magnocellular division of the red nucleus, the perirubral field and in nuclei pontis oralis, pontis caudalis, gigantocellularis and the lateral vestibular nucleus. A moderate AChE activity of the soma and processes is present in the interstitial nucleus of Cajal, the cerebellar fastigial nucleus and the dorsal nucleus of Clarke. A light to moderate AChE activity of the soma and a weak or undetectable activity of the processes is shown by the neurons of the nucleus of the mesencephalic root of V, the dorsal nucleus of the raphae, nuclei annularis, linearis and ventralis of Gudden, the pontine nuclei, the cerebellar dentate and interpositus nuclei and the principal and accessory inferior olivary nuclei. The substantia nigra and locus coeruleus are described elsewhere. (Supported by the Medical Research Council of Canada and the Quebec Health Sciences Council).



AChE-containing neurons of the magnocellular division of the red nucleus (Fig 1), nucleus gigantocellularis (Fig 2), the lateral vestibular nucleus (Fig 3), the fastigial nucleus (Fig 4), and the ventral horn of the spinal cord (Fig 5) in DFP-treated monkeys. Calibration bar is 50μ

906 AN ELECTRON MICROSCOPIC AND GOLGI STUDY OF THE EXTERNAL GRANULAR LAYER IN FETAL MOUSE CEREBELLUM. Jeffrey R. Swarz and Manuel del Cerro. Center for Brain Research, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y. 14642.

The external granular layer (EGL) is the germinal cell layer in the cerebellum that is the precursor of basket, stellate, and internal granule cells. It was the purpose of this study to examine the fetal development of the EGL using the Golgi method and electron microscopy.

The cerebellum of C57BL/6J mice was studied by both light and electron microscopy. Mouse embryos, aged 13-18 embryonic days, were fixed by immersion with 1% glutaraldehyde and 4% formaldehyde or a dimethylsulfoxide-aldehyde mixture. After fixation for two days, the tissue was processed for electron microscopy using standard procedures. Additional fetuses, aged 14-19 embryonic days, were processed using the Stensaas modification of the Golgi method (Stensaas, 1967).

The fetal EGL contains mainly germinal cells and, depending on the age, some neuroblasts in the deepest regions. In addition there are two types of cellular processes: EGL cell processes that are moderately electron dense, have a high ribosomal content and are 5-7 micrometers in length; and Bergmann fibers that are electron lucent, have numerous microfilaments, few mitochondria, some smooth ER and scant free ribosomes. The cell somas are ovoid or irregularly polyhedral, about 8μm in diameter and have a relatively large nucleus that almost fills the central portion of the cell. The nucleus exhibits one or more prominent nucleoli which are heterogenous and have abundant associated chromatin. Other heterochromatin clumps are also preferentially apposed to the nuclear membrane. The cytoplasm surrounding the nucleus is poor in organelles except for abundant free ribosomes. Most of the cytoplasm and cell organelles accumulate at the poles of the soma.

In Golgi preparations horizontal cells with long, irregular processes were observed in the inner layer of the EGL. These cells resemble those described by Cajal (1911) in perinatal animals. Also, granule, basket, Purkinje and Bergmann glial cells could be observed in different stages of development at all fetal ages examined. Additionally, cells in the ventricular layer were present at embryonic ages 14 and 15; these cells had long ascending processes that reached the pial surface. They appeared similar to Bergmann astrocytes identified by Cajal (1911) in the ventricular layer of mice and other species. Unidentified cells were frequently observed juxtaposed to these ventricular cell processes.

These results extend to prenatal ages the ultrastructural findings of del Cerro and Snider (1972) on the EGL. Observations with the Golgi method confirm the existence of Bergmann fibers in the rodent at prenatal ages. They also show that basket cells begin differentiating from the fetal EGL at prenatal ages in rodents as has been shown in primates by other investigators. Lastly, the existence of cells apposed to processes of putative Bergmann astrocytes in the ventricular layer suggests the concept, not previously proposed, that Purkinje cells, Golgi cells and/or neuroblasts of the deep cerebellar nuclei could use these processes as guides in migration to their respective cell layers. (Supported by NIMH grant MH 08034 and NIH grant H-34.6214-JH-1)

907 THE CYTOARCHITECTURE AND FINE STRUCTURE OF THE HYPOTHALAMIC VENTROMEDIAL NUCLEUS. Mark Van Houten* and James R. Brawer* (SPON: Leo Renaud). Tufts University Medical School, Boston, Mass. and McGill University Medical School, Montreal, P.Q.

The hypothalamic ventromedial (HVM) nucleus has been implicated as a neuronal transducer for processes governing feeding and sexual behaviour as well as processes regulating autonomic tone and neuroendocrine activity. Recent biochemical evidence suggests that the varieties of functional specialization within HVM neurons may be manifest at the cytologic level. This report presents the results of a detailed fine structural survey of the HVM of the young adult, sexually mature, male, albino rat. The collection and analysis of data was systematized with reference to a cytoarchitectonic map of the HVM which was prepared prior to the study.

The results of this study reveal a remarkable degree of fine structural variation within the HVM. Furthermore, the use of cytoarchitectonic localization has facilitated a precise description of this heterogeneity with reference to differential, regional patterns of distribution. As many as nineteen different types of neuronal profiles are described with respect to the organizational patterns of the rough endoplasmic reticulum (RER) alone. Pattern variations include, among others, complex honeycomb arrangements and other highly pleomorphic forms involving meandering cisternae of variable lengths. Other profiles are distinguished by the presence of swollen mitochondria which exhibit a paucity of cristae and a blanched matrix, polysomal disaggregation, aberrant nucleolar morphology, and by the presence of annulate lamellae. In addition, many neuronal profiles contain RER-derived myelin figures which are occasionally quite large and numerous, and which probably indicate a focal degradation of cytomembranes. Lipofuscin granules, however, are rare in these young neurons. Cumulatively, various morphologic arrangements among elements of endoplasmic reticulum, polysomes, cytoplasmic filamentous bodies, and annulate lamellae are interpreted as morphologic signs of a rapid turnover of the ribosome-associated cytomembrane system within neurons in which they occur.

HVM glia are also heterogeneous. Many of the HVM astrocytes are organelle-rich and contain numerous, thick fascicles of gliofibrils, glycogen, pleomorphic dense bodies, and myelin figures which may be derived from neighbouring neurons, since they are occasionally observed intercalated between neurons and apposed glial satellites. Signs of degeneration also occur within the neuropil. Large masses of cellular debris are occasionally found within interstitial and perivascular macrophages, while strewn throughout the nucleus are small, spherical masses that appear to be degenerating terminals. These are invariably ensheathed by multiple glial lamellae probably of astrocytic origin. Conversely, varieties of growth cones, indicative of neuronal growth, also are present within the neuropil. This overall morphology suggests the presence within the HVM neuropil of a developmental and/or remodelling process. All of these variations have been localized with respect to the cytoarchitectonic map and it appears that many specific neuronal and glial varieties inhabit distinct regions within HVM. It is anticipated that these region specific variations reflect the functional parcellation of the HVM and that the recognition and localization of cell types will permit further studies into their cytophysiology and role in the varied functions of HVM.

908 DISPLACED NEURAL ELEMENTS WITHIN RAT CEREBELLAR FISSURES. John R. Walker,* Robert L. Stoughton,* Marc D. Palter,* Jeffrey R. Swarz and Manuel del Cerro. Center for Brain Research, School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642.

Clusters of neural tissue have been observed within the cerebellar fissures of untreated and experimentally treated Wistar albino rats and hooded rats.

The observations of these "intra-fissural clusters" were made in conjunction with several series of experiments dealing with the effects of various chemicals on cerebellar morphogenesis. The chemicals used on the treated animals were cyclophosphamide, concanavalin A, cytochalasin B, and RNAse. Control injections for the experiments consisted of Hanks' Balanced Salt Solution, the vehicle for the drug injections. After observing the clusters in these treated animals, we sought to find them in normal, untreated animals, and were successful.

At various ages between 5 and 30 days postnatally, the animals were perfused intracardially with aldehydes. Sagittal slices were cut from the vermis of each cerebellum and embedded in plastic. Optical and electron microscopical studies were undertaken to determine the location, conformation and components of the clusters.

Clusters can show a considerable variation in size. The smallest consist of just a few closely packed cells, while the largest contain many cells and often completely occupy the area between the two folial surfaces.

The most outstanding components of the clusters are identified as external granule cells (EGL), with their characteristic ovoid nuclei containing clumped heterochromatin and surrounded by a thin rim of cytoplasm revealing abundant polysomes and occasional cisternae of rough endoplasmic reticulum. With increasing age a well-developed neuropil is seen with bundles of parallel fibers coursing among the EGL cells, along with occasional Bergmann glial fibers, capillaries and pericytes. A few synaptic contacts and terminals are present in nine day old animals, with numerous well-developed synapses in older animals.

Frequently, pial cells and their processes are interposed between the cluster and the basal lamina of the folial curface. In some cases, the basal lamina is interrupted at the point where the cluster and the parenchyma are continuous. This region, which can be considered the "stalk" of the cluster, consists of EGL cells and Bergmann glial fibers. The Bergmann glial fibers can be seen to penetrate the cluster from the parenchyma. Where the basal lamina is disrupted, it terminates in a meandering, labyrinthine fashion.

The presence of these clusters implies a failure of normal morphogenetic and migrational control mechanisms. Further studies with animals younger than 5 days and older than 30 days are presently under way to search for the earliest occurrence of the cluster and to investigate the question of their persistence and continue differentiation.

(Supported by NIH grants H346214-JHU, MH08034 and MH05405.)

909 ACRIDINE ORANGE FLUORESCENCE MICROSPECTROPHOTOMETRY IN THE ANALYSIS OF NUCLEIC ACIDS IN DIFFERENT MICROENVIRONMENTS AND IN SINGLE NEURONS.
Robert G. Canada* (SPON: T.W. Jenkins). Dept. Biophysics, Coll. Hum. Med MSU, E. Lansing, MI. 48824.

The application of biophysical cytochemistry to single neurons in culture represents a model system whereby inferences, anent the structure and functions of a biopolymer engaged in neuronal interactions, may be devised by studying the interactions of a fluorescent molecular probe and the macromolecule under question. A large segment of this investigation was concerned with the binding and structure characterization of acridine orange -- nucleic acid complexes embedded in gelatin microdroplets, exposed to various microenvironmental conditions. This investigation affirms that acridine orange (AO) has the same binding mode and a specific affinity for each nucleic acid (NA) conformation investigated (rRNA, DNA, Poly U, and denatured rRNA), where each AO-NA complex has a green fluorescence maximum at 536 nm. and a prominent shoulder toward the longer wavelengths at 604 nm. The alteration of the NA micro environment altered the binding of AO to the NA, whereby increasing the AO-NA interaction time, NA denaturation, pH, and dye concentration increased the binding of AO to the NA. In addition, the binding of AO to the NA was enhanced in the presence of NaCl. More importantly, there was a linearity between the AO-NA emission and the amount of NA available for binding an unchanging AO concentration. Fluorescence coefficients for specific staining conditions were procured and employed in the calculation of the NA content inside the soma of single neurons identified in dissociated cell cultures of the rat brain. The NA content per neuron was found to depend more upon neuronal type and maturity than size. The binding of AO to the NA in the neurons and microdroplets is predominantly in the monomer form with a small degree of aggregation.

910 ELECTRON MICROSCOPY OF GOLGI IMPREGNATED TISSUE: A GOLD TONING METHOD.
Alfonso Fairen*, Alan Peters and Julian Saldanha*. Dept. Anat., Boston Univ. Sch. Med., Boston, MA 02118.

Electron microscopy of partially deimpregnated Golgi preparations offers a promising approach to the study of neurons and their relationships. In the simple procedure introduced here the following are emphasized: (1) Sections, 150 µm thick, can be first examined by light microscopy so that impregnated neurons may be classified on the basis of their dendritic and axonal morphology. (2) After deimpregnation and embedding of the material in plastic the neurons are still sufficiently distinct for even fine details such as dendritic spines and axonal boutons to be visible in the light microscope. This facilitates the sampling of specific portions of neurons for electron microscopy. The material used consisted of the brains of young albino rats, fixed by vascular perfusion with buffered solutions containing low concentrations of glutaraldehyde and formaldehyde, and pieces of the brains were impregnated using an osmium-dichromate solution followed by silver nitrate. The pieces were then sectioned with a tissue chopper. The resulting 150 µm sections containing selected neurons were gold-toned in gold chloride followed by oxalic acid and the initial silver chromate impregnation deposit was removed with sodium thiosulfate. After embedding in plastic, thin sections examined in the EM show deimpregnated neurons to have a deposit of tiny grains of gold beneath their plasma membranes. The fine structure of the deimpregnated neurons is well preserved, so that cytological details can be discerned and synapses are readily apparent.

Supported by a PHS International Fellowship (1 FO5 TWO2275) to A.F. and by NINCDS grant NS-07016.

911 DEVELOPMENT OF THE NERVOUS SYSTEM IN THE HORSE LEECH. Juan H. Fernandez*. Dept. Mol. Biol., Univ. California, Berkeley 94720 and Dept. Anatomy, Case Western Reserve Univ. School of Medicine, Cleveland, Ohio 44106 (Spon: R. L. Calabrese)

The ventral nerve cord of the leech Haemopis marmorata originates in the embryo from stem cells lining the medial surface of its germinal bands. These cells form three longitudinal series of ganglionic primordia, two paired lateral and one unpaired medial, from which the nerve cord develops in a rostro-caudal sequence. Each abdominal ganglion arises by fusion of two lateral and one medial primordia lying in register. The medial primordia appear first and consist of few cells whereas the lateral primordia appear later and consist of many cells. The pair of lateral primordia are linked by the medial primordia in a ladder-like fashion. The infrapharyngeal and caudal ganglia form similarly by the fusion of twelve and twenty one primordia respectively. The primordial neuroblasts contain a large nucleus, a prominent nucleolus and a thin rim of cytoplasm rich in free ribosomes. Growth of their processes begins before fusion of the primordia is completed. Their cytoplasms become gradually denser, probably because of accumulation of rough endoplasmic reticulum and other organelles. Axons grow into the nascent ganglionic neuropile which at this stage consists of two separate bilateral compartments. The earliest axon pathways are those forming the interganglionic connectives and the commisures linking the neuropile compartments. Developmental anomalies such as misalignments or complete absence of extensive parts of the nerve cord occur in nature at surprisingly high frequencies. A developmental model under which the nerve cord arises from the descendants of a single pair of germinal cells accounts for the results. (Supported by the Guggenheim and Sloan Foundations, NIH Grant NS 07403-15 and NSF Grant BMS 74-24637.

912 THE PRIMARY OLFACTORY NEURON: A SHORT LIVED, DISPOSABLE NEURON IN THE VERTEBRATE NERVOUS SYSTEM. P.P.C. Graziadei & G.A. Monti Graziadei*. Dept. Biol. Sci., Florida State Univ., Tallahassee, FL 32306.

Recent evidence indicates that the olfactory primary neuron turns over during the entire life of mammals and lower vertebrates (Graziadei & Metcalf, Z. Zell. 116:305, 1971; Graziadei, Tiss. Cell 5:113, 1973; Graziadei & Graziadei, J. Neurocytol. 5:11, 1976). New synaptic endings, consequently, are continuously reformed in the olfactory bulb's glomeruli. The life span of these neurons in mice has been estimated, by means of autoradiography, to be in the range of 4 weeks (Graziadei & Graziadei, Handbook Sensory Physiol., Vol. IX, 1976; Moulton, D. G., Ann. N.Y. Acad. Sci. 237:52, 1974). Morphological evidence of the developmental changes of the neurons, from the stage of indifferentiated basal cells to the stage of senescent degenerating elements, will be presented. The changes repeat, during the short period of 30 days, the basic structural patterns observed in other neurons of the nervous system that extend for the entire life of the animal.

This research was supported in part by USPHS NS08943.

913 ULTRASTRUCTURAL AND FUNCTIONAL CONSIDERATIONS OF AREA POSTREMA EPENDYMA IN MAMMALS. Peter M. Klara and Kenneth R. Brizzee Delta Regional Primate Research Center and Department of Anatomy, Tulane University School of Medicine, New Orleans, La. 70112.

Ultrastructural investigations of the area postrema (AP) in several mammalian species have revealed morphological characteristics that distinguish these cells from adjacent mural ependyma. The absence of kinocilia in AP ependyma of the squirrel monkey, dog and cat, is strikingly contrasted with abundant kinocilia seen in surrounding mural ependyma. Microvilli are prominent in all species examined and appear to be concentrated at the boundary between ependymal cells. Also, supraependymal cells are sometimes observed in association with AP ependyma. While all three species demonstrate similarities the cat ependmal cells have features that distinguish them from both the dog and squirrel monkey. Ependymal cell bodies in direct contact with the ventricular lumen also demonstrate direct contact with perivascular spaces. Slender processes from ependymal cells appear to extend into the AP parenchyma. These processes exhibit both lumenal and perivascular contacts reminiscent of hypothalamic tanycytes bordering on the third ventricle. While tanycytes are usually associated with CNS areas of known secretory activity such a function has not been established for the AP. However, AP morphology, like that of other circumventricular organs such as the median eminence, suggests secretory activity. Ultrastructural histochemistry has been undertaken in an attempt to assess enzymatic activity. The role of the ependyma in overall AP function will be discussed in light of these findings and current theories concerning the function of circumventricular organs.

This research was supported by NASA-Ames Grant NS6 2139 and NIH Grant RR00164-14.

914 FINE STRUCTURAL STUDY OF SYNAPTOGENESIS IN THE CHICK OPTIC TECTUM. Catherine F. McGraw and Barbara J. McLaughlin. Dept. of Anat., UTCHS, Memphis, Tenn. 38163.

Synaptic junction formation has been studied in the rostral pole of the chick optic tectum at all days from embryonic day 6 through hatching, and in the 5 day hatchling. At embryonic day 6 desmosome-like junctions or puncta adhaerentia are observed between developing profiles in all layers. These junctions are seen throughout development but there appear to be fewer present in the later stages. Coated vesicles are also observed throughout development in neuronal soma, neurites, and glial elements. Some coated vesicles appear to be in continuity with the plasmalemma adjacent to puncta adhaerentia and in the vicinity of the postsynaptic densities of developing synaptic contacts. Unlike previous studies which suggested that the earliest synapses in the chick optic tectum are formed at embryonic day 11 (Cantino and Sisto-Daneo, Experientia 29: 85, 1973; Rager, Proc. Roy. Soc. London 192: 353, 1976), immature synaptic terminals are found in the superficial layers of the developing tectum by embryonic day 7. These terminals contain only a few vesicles and are forming synapses primarily on developing dendritic profiles. After embryonic day 15 and in the hatchling, mature synapses containing many synaptic vesicles are observed making axodendritic, axosomatic, and axospinous contacts. The contralateral optic fibers reach the rostral pole of the chick optic tectum by embryonic day 6 (DeLong and Coulombre, Exp. Neurol. 13: 351, 1965); therefore, it is possible that some of these early forming synapses are retinotectal projections. This possibility may be tested by eye enucleations and tracer studies of the developing retinotectal system. (Supported by USPHS Grants 5T01-GM00202 and RR-05423.)

915 CORRELATIVE SCANNING-TRANSMISSION ELECTRON MICROSCOPY OF THE THIRD CEREBRAL VENTRICLE OF THE MALLARD DUCK (ANAS PLATYRHYNCHOS). Thomas H. McNeill* (SPON: N.H. McArthur). Dept. Anat., Univ. Rochester Sch. Med., Rochester, NY. 14642.

The third ventricle of three male and two female adult mallard ducks were analyzed by the combined Scanning/Transmission electron microscopic (SEM/TEM) technique. The dorsal wall of the third ventricle is characterized by an abundance of ciliated ependyma. The cilia which obscure the underlying substructure are uniform and homogenous and only occasionally display small dilations at their apical tips. The organum vasculosum of the lamina terminalis (OVLT) is demarcated by an abrupt termination of the ciliated ependyma. The ventricular surface of the OVLT is characterized by numerous microvilli and apical bleb-like excrescences. Occasionally single cilia are found on the ventricular surface. The luminal surface of the median eminence (ME) closely resembles that of the OVLT. However, the apical blebs were more numerous and concave in appearance. The paraventricular organ (PVO) resembled a bilateral groove-like formation amongst the ciliated ependymal cells of the lateral ventricular wall. Dense bleb-like protrusions and microvilli project into the ventricular lumen. In contrast to the mammalian ventricular system, a subpopulation of supraependymal cells in the supraoptic, infundibular, or mammillary recess could not be found. However, a few cells similar to the Type 2 histiocytes were localized at the most caudal end of the ventricle adjacent to the cerebral aqueduct. A distinct nonciliated morphological correlate of the subcommisural organ (SCO) was also absent.

(Supported by USPHS Program Project Grant NS-11642.)

916 ULTRASTRUCTURAL MORPHOLOGY OF THE ADULT DOG AREA POSTREMA M. Carmen Palazzo and Kenneth Brizzee (SPON: C.A. Roberts) Dept. of Anatomy, Tulane Medical School, New Orleans, La. 70112.

The Area Postrema (AP), a highly vascular organ situated near the obex, has been postulated to participate in an number of physiological roles. The most substantiated hypothesis, that the AP is involved in the emetic response was first proposed by Borison and Brizzee (1951). This has since been corroborated by various experiments in which both lesioning techniques and drug testing were used. The dog will respond with an emetic response to both drug and motion stimuli by four weeks of age, thus making it an excellent experimental model for motion sickness experiments. The ultrastructure of this organ has been described in a variety of animals including the squirrel monkey, cat, rat, rabbit and mouse. The present study was conducted in an effort to determine if the adult dog AP differs in morphology from this organ as previously described in these other mammalian species. The structures which are characteristic of the AP in most mammalian species that were also observed in the dog are the presence of both neuronal and glial elements, ependyma with microvillar projections, perivascular spaces delineated from the AP parenchyma by a well defined basal lamina, infolded nuclear membrane and honeycomb nucleoli.

Supported by NASA-Ames Grant NSG 2139 and NIH Grant RR00164-14.

917 EVIDENCE OF NEUROENDOCRINE FUNCTION IN SPINAL CORD. Terry J. Sims* (SPON K. IKEDA). Div. Neurosciences, City of Hope Med. Ctr., Duarte, CA. 91010.

The presence of specialized cerebral spinal fluid (CSF) contacting neurons within the spinal cord of a number of species has led to the speculation that these neurons are sensitive to substances in the CSF. To investigate more fully the nature of these neurons in the Salamander Ambystoma mexicanum, a series of experiments was conducted utilizing fluorescence histochemistry, as well as light and electron microscopy combined with the administration of either 6-OH dopamine (6-OHDA) or gonadotropin. Results from these experiments have shown that at least two distinct types of CSF-contacting neurons exist within the spinal cord. Type 1 neurons are located in or near the dorsal half of the ependymal zone, and they respond to gonadotropin administration. This response consists of marked increase of: a) the Golgi apparatus b) the endoplasmic reticulum and c) Herring body-like inclusions within the apical cytoplasm and processes contacting the lumen of the ventricle. Type 2 CSF-contacting neurons are located in or near the ventral half of the ependymal zone, and they do not exhibit an apparent response to gonadotropin administration. Furthermore, type 2 CSF-contacting neurons characteristically contain a catecholamine as determined by histofluorescence and intraventricular 6-OHDA administration. In addition to a ventricular process, type 2 neurons also possess ventrally-oriented processes that terminate in expansions embedded within the glia limitans overlying the ventral spinal artery. The ultrastructural appearances of type 1 CSF-contacting processes and type 2 ventral processes are suggestive of a neurosecretory function. These experimental observations in combination with other studies in progress demonstrate that at least one population of spinal cord CSF-contacting neurons is sensitive to alterations in hormonal levels, and they further implicate the CSF in the transport of bioactive substances. (Supported by USPHS grant #NS09578).

918 THE DISTRIBUTION OF PHOSPHOTUNGSTIC ACID STAINING IN OLIGODENDROCYTES DURING CNS MYELINATION. Takeshi Tabira* and Henry deF. Webster. Lab. Neuropath. Neuroanat. Sci., NINCDS, NIH, Bethesda, MD. 20014.

Ethanolic phosphotungstic acid (E-PTA) has been used extensively to investigate the cytochemistry of synaptic membranes. To study junctional complexes and other membrane specializations in myelin forming cells, optic nerves of Xenopus tadpoles were stained en bloc with E-PTA (Bloom et al., 1968). Heavily stained desmosome-like junctions were found between paranodal terminal loops but tight junctions and terminal bars were not stained. Beneath inner membrane leaflets of oligodendrocyte tongue processes and paranodal terminal loops, there was a moderately dense layer of PTA staining that extended to compact myelin's major dense line. This undercoating also extended along inner leaflets where inner or outer spiral layers of myelin were still separated by oligodendrocyte cytoplasm. In compact myelin, the major dense lines were stained lightly; the intraperiod lines and all other membranes were translucent. Intensely stained microtubules (usually 1-4) were also found in tongue processes and terminal loops of oligodendrocytes. They were located along inner membrane leaflets close to or in contact with the undercoating described above. Microtubules in axons and other cells were very lightly stained. Finally, when sections of adult Xenopus optic nerves were studied, the membrane undercoating and microtubules of oligodendrocytes were less densely stained by PTA than those observed in tadpoles. These observations suggest that during CNS myelin formation, PTA positive substances are distributed along oligodendrocyte membranes and also are associated with microtubules found in their processes. Similar studies of other CNS regions and of other species are in progress.

919 THE SYNAPTIC SPINULE IN DENDRITIC SPINE SYNAPSES OF THE HIPPOCAMPAL DENTATE GYRUS. Sally Tarrant* and Aryeh Routtenberg (SPON: T.J. Marczynski), Cresap Neurosci. Lab., Northwestern Univ., Evanston, Illinois, 60201.

The synaptic spinule: (a) is an invagination of the pre-synaptic terminal by pre- and post-synaptic membranes and cytoplasm of the post-synaptic process; (b) is present within an interruption in the post-synaptic specialization; (c) is seen in dendritic spine synapses in rat dentate gyrus; (d) is associated with a spine apparatus in the spine cytoplasm and (e) has coated vesicles attached to the axonal membrane component.

The previously reported spinule complex (Westrum and Blackstad, J.comp. Neurol.119 (1962) 251), micropinocytotic invagination (Andres, Z.Zell.64 (1964) 63) "W" synapse (Cotman, et al. Br. Res.63 (1973) 205) and synapse with perforated post-synaptic plate (Peters, Z.Zell.100 (1969) 487) are considered to be instances of synaptic spinules.

We have observed no significant increase in the occurrence of the synaptic spinule or spine apparatus as a function of fixation delay (Routtenberg and Tarrant, Tiss. & Cell 6 (1974) 777). The rate of occurrence of these features is unaffected by the administration of short-term (5 min) anesthesia.

The absence of the synaptic spinule on certain terminals in the subgranular hilar area is reported. These terminals are suggested to contain biogenic amine neurotransmitters and to terminate upon interneurons.

It is concluded that the presence of synaptic spinules in dendritic spine synapses, one per 27 μ^2 of hippocampal dentate gyrus molecular layer, indicates that it is a significant component of these synapses. Its close association with the "active zone" is suggestive of synaptic spinule participation in synaptic functioning. Supported by The Alfred P. Sloan Foundation, NS 10768, MH 25281 and BMS 19481 grants to A.R.

Neuroendocrinology

920 CATECHOLAMINE SENSITIVE ADENYLATE CYCLASE IN RAT, GUINEA PIG AND MONKEY HYPOTHALAMUS. Ho Sam Ahn* and Maynard H. Makman* (Spon: Kinuko Suzuki), Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Hypothalamic adenylate cyclase of rat, guinea pig and monkey was studied employing ATP dependent formation of cyclic AMP in hypothalamic homogenates. Adenylate cyclase (AC) of each of the above species was found to be stimulated by catecholamines, NaF and GPP(NH)P, with the latter being the most potent agent. AC was also stimulated by histamine in guinea pig but not in rat or monkey. In monkey hypothalamus, cyclic AMP formation was linear up to 5-10 min in the presence and absence of agonist and proportionally increased with increasing concentration of the enzyme up to about 60 μg protein/0.1 ml. The relative potency of catecholamines in stimulating the cyclase enzyme was dopamine (DA) > norepinephrine $\geq$ epinephrine > isoproterenol although the maximum stimulation for each catecholamine was achieved at about the same concentration (30 μM). AC of both Cebus and rhesus hypothalamus was stimulated by not only DA but also other DA-receptor stimulating agents such as apomorphine and 1-(3,4-dihydroxybenzyl)-4-(2-pyrimidinyl)-piperazine (S584) (relative potencies: apomorphine $\geq$ DA > S584). The stimulation of hypothalamic AC activity by DA was effectively antagonized by fluphenazine, a potent DA-receptor blocking agent. Activity of monkey median eminence was also stimulated by DA and this stimulation antagonized by fluphenazine. These results indicate presence of DA-specific AC system in hypothalamus and median eminence. The relative potencies of DA-receptor blocking agents in antagonizing DA stimulation of hypothalamic AC was as follows: Fluphenazine > clozapine > thioridazine, pimozide $\gg$ haloperidol. This is the first report of catecholamine-sensitive AC in cell free hypothalamic preparations. The requirement of small amounts of tissue (>25 μg protein/tube)for assay will facilitate characterization of catecholamine receptors associated with AC in discrete regions of hypothalamus.

921 BICENTENNIAL HISTOFLUORESCENCE IN A COLONIAL AVIAN. A. Armer*, J. R. Sladek, Jr., B. Franklin*, Y. Cheung*, D. Kent*, G. Hoffman and T. Jefferson* (SPON: John Hancock). Dept. Anat., Univ. Rochester Sch. Med., Rochester, NY. 14642.

When in the course of examining monoamine histofluorescence in the brain of phasianus colchicus (New York State ring neck pheasant) we (the people) discovered a unique and heretofore unreported histofluorescence which contained red, white, and blue hues. This serendipitous bicentennial mixture heralded further examination of this revolutionary finding.

Specific interest centered upon the pineal gland of this species. Pineals were dissected from decapitated specimens and prepared for either electron microscopy or formaldehyde-induced fluorescence of monoamines. The parenchyma of the gland consisted of columnar cells arranged around a lumen, giving the gland a distinct follicular appearance. These cells displayed a prominent Golgi apparatus, abundant mitochondria, microtubules and granular-ER. At the periphery of the follicles, the cells rested upon a distinct basement membrane, outside of which were blood vessels, nerve fibers and various connective tissue elements. The terminals of pinealocyte processes contained both clear and dense-cored vesicles. The apical surfaces of many of these cells were characterized by surface modifications which resembled outer segments of photoreceptive cells.

Catecholamine containing fibers displaying a blue hue were seen surrounding the parenchyma of this semi-lobulated gland. Microspectrofluorometric analysis revealed catecholamine excitation and emission spectra at 410 and 480nm respectively. This fluorescence appeared white upon extended photographic exposure. This catecholaminergic distribution presumably arises from superior cervical ganglia as is the case in other species, both avian and mammalian. In addition to this expected fluorescence, an intense red fluorescence was seen within the parenchyma of individual lobules. This red fluorescence demonstrated an extremely fast excitation fade time, with an initial quenching within 2 seconds of exposure followed by a near complete fading at 12-15 seconds. Emission spectra revealed a bi-modal emission peak at 635nm and 665nm (A). After exposure to blue light the primary peak at 635nm decreased markedly, while the peak at 665nm increased (B). Excitation maxima occurred at 430 and 480nm. When the scanning diaphram was enlarged to include nerve fibers surrounding the lobules emission peaks characteristic of catecholamines appeared (C).

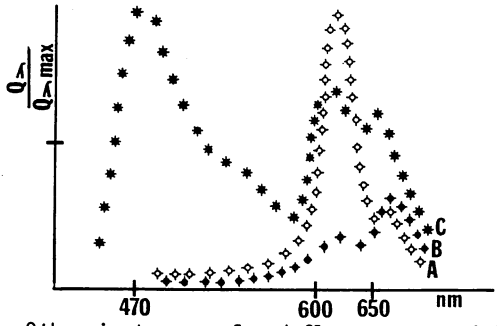


Figure 1

Emission spectra of pinealocytes and nerve fibers.

Other instances of red fluorescence with rapid fade times have been reported in the harderian gland and possibly the retina. We speculate that some type of rudimentary visual pigment may be present in this vestigial light sensitive organ and that the localization of this substance may be within the retinal type ultrastructural characteristic of pheasant pinealocytes. (Supported by USPHS Program Project Grant NS-11642)

922 MORPHOLOGICAL CHANGES IN HYPOTHALAMIC NEUROSECRETORY NEURONS DURING THE DIURNAL CYCLE. W.E. Armstrong* and G.I. Hatton. Dept. Psych., Michigan State University, East Lansing, MI 48824.

Discoveries of direct retinal projections to the suprachiasmatic (SC) nuclei and the localization of arginine vasopressin (AV) within SC prompted quantitative light microscopical examinations of nucleolar and cell size changes in these nuclei and in neurons of the supraoptic anterior (SOa), circularis (C), lateral (PVl) and medial paraventricular (PVm) nuclei in rats killed at either lights on (Lon) or lights off (Loff) after being on a 12:12 light-dark schedule for 30 days. These times were chosen to approximate known diurnal peaks and troughs in CRF-ACTH-corticosterone outflow and pituitary AV. The brains of 8 rats (4 per group) were embedded in paraffin, sectioned at 6μm, and stained with thionin. For nucleolar counts, random samples of 100 or 150 cells were taken from SOa, PVl, PVm, dorsomedial SC (SCdm), ventrolateral SC (SCvl), and C from each rat. SC was divided in an attempt to separate AV-containing cells (in SCdm) from non-AV-containing cells (in SCvl). Cell size measurements were made on 60 cells per rat. The variables of interest in the magnocellular cells were a) % cells with 2 or more nucleoli; b) % cells with a single nucleolus which was in contact with the nuclear membrane (marginated); c) cell size. In addition to the above, SC neurons were further divided to obtain a) % cells whose nucleus exhibited a prominent invagination; and b) % cells with a single nucleolus in contact with this invagination. All results cited were statistically significant.

PVl and PVm exhibited greater cell areas at Loff than at Lon, with the cells of PVl always greater than those of PVm. SOa showed a marginal change in this same direction and C did not change. While neither SCdm or SCvl appeared to change, coronal plane measurements of SCdm + SCvl showed greater cell areas at Loff than at Lon. SOa was the only nucleus whose % 'multiples' changed across times, with a profound increase at Loff ($\bar{x}$=31%) over Lon ($\bar{x}$=13%). PVl had consistently more 'multiples' than PVm, and more marginated single nucleoli than PVm. There were no differences in margination in the magnocellular neurons between Lon and Loff.

In SCvlm the % invaginated cells was greater at Loff than Lon. In SCdm a majority of cells were invaginated and these cells had more 'multiples' than non-invaginated cells at Loff. SCdm had more non-invaginated neurons which exhibited marginated single nucleoli than SCvl. A greater % invaginated cells with a single nucleolus in contact with the invagination occurred in SCvl compared to SCdm. At Lon, SCvl had a greater % of cells with single nucleoli in contact with the nuclear membrane than did SCdm. These subtle differences between SCdm and SCvl indicate the two populations may be functionally disparate and characterized the diurnal response of SC at the times measured. Our hypothesis that invagination facilitates nucleolar contact with the nuclear membrane (and thus cytoplasm) was supported by comparing invaginated cells with single nucleoli in contact with the nuclear membrane and non-invaginated singles, the former ($\bar{x}$=82.9%) always greater than the latter ($\bar{x}$=64.7%) across subnuclei and conditions.

As a whole, these results suggest phase differences between PV and SOa, SOa and C, and SCvl and SCdm. There was no clear indication that the AV-containing cells of SCdm behave more like neurosecretory cells than the cells of SCvl. The difference in SOa from Loff to Lon supports the previous description of pituitary AV shifts, but it is difficult to compare the magnitudes of the changes. Further studies are in progress to complete the diurnal rhythm for all nuclei listed. The findings serve to point out to students of neurosecretory cell morphology the structural changes occurring throughout the diurnal cycle.

923 CORRELATIVE SEM-TEM STUDY OF THE RAT MEDIAN EMINENCE AND THE HYPOTHALAMIC WALL OF THE THIRD CEREBRAL VENTRICLE. Nabil A. Azzam and John C. Herr*. Dept. Anat., Univ. Of Iowa, Iowa City, Ia. 52242

The median eminence and the lateral wall of the third ventricle have been extensively studied not only by the light microscope but also by the scanning and transmission electron microscopes. Recent microanalytical techniques have confirmed the presence of hormones and neurohormones in the cerebrospinal fluid (CSF); however the site and mode of secretion into and manner of their removal from the CSF and subsequent transport to the target organ is unknown. Ependymal cells and specifically tanycytes have been hypothesized to play a role in the regulation of neurohormones within the CSF and in light of this, we have examined with SEM and TEM their cytological features, in particular, their surface specializations. In an attempt to avoid the reported changes that occur in ependymal cells during the estrus cycle, only normal adult male rats were utilized in this investigation. Although no attempt as yet has been made to thin section blocks already scanned, we have carefully correlated comparable regions of the ventricular surface.

The cells lining the surface of the median eminence and the lower 1/3 of the lateral wall of the third ventricle inferior to the massa intermedia, exhibit surface modifications that range from non-ciliated type ependymal cells to cells with slender microvilli or short expanded microvilli, as well as exhibit an accumulation of blebs of varying diameter. Based on cytoarchitecture, three varieties of blebs have been observed. The first type is entirely filled with whorles of smooth endoplasmic reticuluum. The second type contains a homogeneous cytoplasm virtually devoid of organelles. The third type is filled with a flocculent material more lucent than the adjacent cytoplasm. Only an occasional single cilium is observed in these cells and this contrasts to the heavily ciliated cells of the upper 2/3 of the lateral wall of the third ventricle. Certain of the short expanded microvilli contain membrane bounded granules, 300-350 nm in dia. These were observed adjacent to slender microvilli from the same cell. A number of supra-ependymal neurons were observed on the surface of the median eminence. We have observed classical tanycytes extending from the ventricular surface to surround capillaries with their foot processes.

924 NEUROTENSIN: CENTRAL NERVOUS SYSTEM EFFECTS OF A HYPOTHALAMIC PEPTIDE.
G. Bissette*, C.B. Nemeroff, P.T. Loosen*, T.S. Barlow*, A.J. Prange, Jr.* and M.A. Lipton. Dept. Psychiatry and The Neurobiology Program, Biol. Sci. Res. Ctr., Univ. North Carolina, Chapel Hill, N.C. 27514

It is now established that many of the naturally occurring peptide hormones have behavioral and pharmacological actions which are distinct from their classical endocrine functions. Thus, work from this laboratory has shown that intraperitoneally (IP) or intracisternally (IC) administered thyrotropin-releasing hormone (TRH) will antagonize the sedation and hypothermia induced by a variety of central nervous system depressants, including barbiturates. While screening various peptide hormones and analogues for such analeptic activity, we tested IC and IP administered neurotensin in the pentobarbital antagonism test. Neurotensin is a recently isolated hypothalamic tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-COOH) reported to be present in the mouse, rat, rabbit and human. The IC administration of neurotensin (0.1-46µg) to mice ten minutes after injection of sodium pentobarbital (50 mg/kg IP) resulted in a marked dose-related increase in sleeping time, determined as the interval between loss of the righting reflex and its subsequent recovery. In addition, the incidence of mortality after this usually nonlethal dose of pentobarbital was markedly enhanced after IC neurotensin. Peripherally administered neurotensin (4.6 mg/kg IP) failed to alter barbiturate-induced sedation. In an attempt to define the mechanism of this apparent synergistic interaction between pentobarbital and neurotensin, the disposition of IP ^{3}H-pentobarbital after IC neurotensin (30 µg) was examined. Neurotensin appeared to significantly decrease the rate of metabolism of ^{3}H-pentobarbital in plasma, liver, and brain. Since it is well established that body temperature can alter the rate of barbiturate metabolism, we studied the effects of IC or intravenous (IV) neurotensin on thermoregulation in both ambient (25 °C) and cold (4 °C) environments. Although IV administered neurotensin (10,30,100 µg/mouse) had no discernible effect on thermoregulation, the IC administration of this peptide markedly reduced the body temperature of mice at both 25 °C and 4 °C when compared with control animals. In addition we noted that the IC administration of TRH, luteinizing hormone-releasing hormone, melanocyte stimulating hormone-release inhibiting hormone (MIF I), somatostatin, and substance P had no effect whatsoever on ability to thermoregulate in a cold environment. Further evidence of a central nervous system effect of neurotensin was the observation that infusion of 30 µg of this peptide into the lateral ventricle of unanesthetized rats significantly reduced locomotor activity when compared with saline-treated controls. Locomotor activity was assessed by the use of electromagnetic activity cages. This concatenation of findings indicate that neurotensin, a hypothalamic peptide, can exert direct central nervous systems effects and may play a role in thermoregulatory processes.

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925 SEPTAL AREA CONTROLS GROWTH IN HAMSTERS. Katarina T.Borer,Robert P.Kelch, Mary S.White*,Lynmarie Dolson* and Lawrence L.Kuhns. Neuroscience Lab., Dept.Pediat.and Dept.Radiol.,Univ.Michigan,Ann Arbor,MI.48104.

A report (Hobbs et al.,Physiol.Behav.9:349,1972) that septal lesions accelerate weight gain in adult hamsters suggested the possibility that this brain area may play a role in the neuroendocrine control of growth in this species. To test this hypothesis lesions were placed in precommissural septum in adult female hamsters of which 52 had intact pituitaries (INT) and 13 were hypophysectomized (HYPEX). Fiftyone other INT and 13 HYPEX hamsters underwent sham surgery. Ponderal and linear growth was monitored by daily weight measurements, by length measurements on days 0 and 76 post-surgery, and by caliper measurements of bone lengths from radiographs of hamster skeleton obtained on day 76. In addition, percentage of body fat was determined from body water content in hamsters on day 76 post surgery. Endocrine changes were examined by measuring the concentration of growth hormone (GH) in the serum with a rat GH radioimmunoassay (RIA) kit (a gift from NIAMDD) and concentration of serum insulin (I) with a heterologous RIA with a rat insulin standard (a gift from Eli Lilly Co). A difference in the slope of the rat GH standard and of the hamster anterior pituitary inhibition curves necessitated running in each assay a standard curve prepared from serial dilutions of a standard hamster anterior pituitary (SHAP) and expressing serum GH concentrations as μg of SHAP.

In INT hamsters septal lesions led to tenfold acceleration of ponderal growth rate and to significant elongation of skull, vertebral column, humerus,and femur. Septal lesions were also associated with significant increases in food intake (11.52±0.18 vs 9.88±0.14 gm/100 gm body weight/day), in concentration of GH in the serum (122.13±35.57 vs 43.68±8.56 μg SHAP/ml) and in concentration of I in the serum of 4-hr-fasted (7.11±1.89 vs 2.55± 0.24 ng/ml) and of ad-libitum-killed hamsters (16.99±4.08 vs 6.69±1.41 ng/ml).The percentage of body fat in septal-lesioned and sham-lesioned hamsters was not different.

In HYPEX hamsters septal lesions did not induce significant changes in the rate of weight gain, skeletal length, percentage of body fat, amount of food eaten, or concentration of GH and I in the serum.

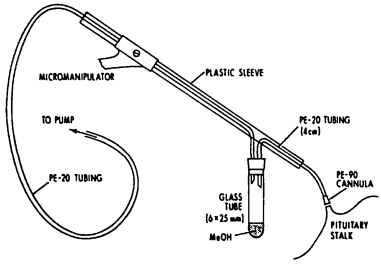
These data indicate that septal area or the adjacent fibers of passage exert tonic suppression over ponderal and linear growth in adult hamsters which have entered the asymptotic phase of growth.

926 A PONTINE-HYPOTHALAMIC PROJECTION IMPLICATED IN CONTROL OF THE RELEASE OF ACTH. D.E. Carlson*, J.W. Maran*, D.G. Ward*, D.S. Gann. Dept. Biomed. Engr., The Johns Hopkins University Sch. of Med., Baltimore, Md. 21205

Both the medial dorsal posterior hypothalamus (MDPH) and the dorsal rostral pons (DRP) have been implicated in control of release of ACTH. We have investigated the possibility that cells in MDPH are activated from the ACTH-active pontine region. Fourteen cats were anesthetized with chlorolose-urethane and immobilized with gallamine. Regions in DRP were stimulated (100-800 μa pulses, 50 μsec, 1/sec) and single cells were recorded in hypothalamus (stainless steel microelectrodes, 2μ tip). Forty-eight cells were studied in medial posterior hypothalamus, of these, 19 were driven orthodromically by stimulation of DRP. Of 13 cells studied in MDPH and driven from DRP, all were facilitated. In contrast, of 6 cells studied in a region ventral to MDPH and driven from DRP, 5 were inhibited. The difference in the responses of dorsal and ventral regions to stimulation of DRP was significant ($P<0.01$). Of the 13 cells in MDPH driven from DRP, 7 were driven from ACTH-active sites in DRP. Five of these cells were in regions of MDPH where stimulation leads to decreases in ACTH; 2 were in regions of MDPH where stimulation leads to increases in ACTH. Regions of DRP stimulating ACTH were connected to regions of MDPH stimulating ACTH, and conversely, regions of DRP inhibiting ACTH were connected to regions of MDPH inhibiting ACTH for 6 of the total of 7 cells ($P<0.05$). The results indicate the presence of projections from DRP to posterior medial hypothalamus, with stimulatory connections principally in medial dorsal hypothalamus and inhibitory connections principally in medial ventral hypothalamus. The results suggest that ACTH-active areas in the dorsal rostral pons may control ACTH-active areas in the medial dorsal posterior hypothalamus through direct excitatory pathways for regions leading both to stimulation and inhibition of release of ACTH. Since cardiovascular information is conveyed to ACTH-active regions both in the dorsal rostral pons and in the medial dorsal posterior hypothalamus, one or more pathways mediating hemodynamic control of release of ACTH may pass through the dorsal rostral pons. (Supported by NIH grants AM14952, GM00576, GM07031, and GM00836)

927 MEASUREMENT OF THYROTROPIN RELEASING HORMONE (TRH) ACTIVITY IN PITUITARY PORTAL BLOOD OF RATS. M. Ching* and R.D. Utiger* (SPON: L.G. Abood). Dept. Anat., Univ. Rochester Sch. Med., Rochester, NY. 14642 and Dept. Med., Univ. Penn. Sch. Med., Philadelphia, PA. 19174.

A method has been developed using methanol for the rapid preservation and extraction of endogenous TRH from pituitary portal blood. Normal male Sprague-Dawley rats, weighing 400-500 g, were anesthetized with 42.5 mg α chloralose-425 mg urethane/kg BW and portal blood collected using a parapharyngeal approach (Porter & Smith, Endocr. 81:1182, 1967). Following completion of the surgical preparation, the rat was injected iv with 100 USP U heparin and the pituitary stalk transected. The portal blood was collected by 2 methods. In one method (group 1, 10 rats) the proximal cut end of the stalk was cannulated and portal blood withdrawn at a rate of 7.9 µl/min into a coil of polyethylene PE-20 tubing (inside dia of 0.38 mm) that was immersed in an ice bath. The blood was cooled to 6°C within 7-9 min and maintained at this temperature for the duration of the collection period of 1-2 h. In the second method (group 2, 5 rats) portal blood was withdrawn at the same rate but into a 4 cm length of PE-20 tubing where, within 20-30 sec, it mixed with 200 µl of 100% methanol in a glass reservoir (Fig. 1). In this group the alcohol-blood mixture remained at room temperature (22°C) for the duration of the collection period. The rat was then decapitated and trunk blood collected and plasma extracted with methanol 20 min later (group 1) or the blood mixed with 2 volumes of methanol within 5 sec (group 2). In group 1, hematocrit determinations were made on small aliquots (50-100 ul) of the portal and peripheral blood. Portal blood exhibited an average hematocrit value of 40% which was nearly equal to that of peripheral blood (47%). The volume of blood extracted directly into methanol (group 2) was estimated by weighing the dried blood precipitate and calculating that 70-80 mg = 1 ml. Samples of blood or blood extracts were centrifuged at 1500 x g for 15 min at 6°C and the alcoholic supernatants or plasma mixed with 2 volumes of 100% methanol. The mixtures were then centrifuged and the supernatants evaporated to dryness at 37°C under N_2 gas. The residues of portal and peripheral blood extracts were reconstituted in 0.2-0.4 ml PBS (0.01 M PO_4, 0.15 M NaCl, pH 7.5) containing 0.25% BSA then assayed for TRH (Bassiri & Utiger, Endocr. 90: 722, 1972). Recovery of synthetic TRH (Guillemin) added to 3 samples of peripheral blood-methanol mixture was 80%. Aliquots of portal and peripheral plasma from rats of group 1 that were not extracted with methanol were assayed for T_4 (Chopra, JCEM 34:938, 1972).



The results show that endogenous TRH was not detected ($<$ 20 pg) in paired samples of portal (100-200 µl) and peripheral (1 ml) plasma obtained from rats in group 1. In this group, the concentration of T_4 in portal plasma was 20% that of peripheral plasma (mean $\pm$ SEM of 0.4 $\pm$ 0.2 vs 2.1 $\pm$ 0.1 µg/100 ml, $p < 0.001$). In group 2, portal blood concentration of TRH, not corrected for recovery, was 440 $\pm$ 30 pg/ml and the trunk blood concentration was 40 $\pm$ 20 pg/ml ($p < 0.001$). This is the first unequivocal demonstration of TRH in portal vein blood.

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928 PERSONALITY CORRELATES OF PLASMA TESTOSTERONE AND 17B-ESTRADIOL IN COLLEGE MALES AND FEMALES. Reid J. Daitzman, Dept. Psych., Sch. Med., U. of Virginia, Charlottesville, VA.22901.

The relatively recent development of biochemical techniques sufficiently sensitive and reliable to measure circulating levels of the sex hormones androgen and estrogen has initiated research into the area of human psychoendocrinology. The present research investigated whether differences in natural levels of plasma androgens and estrogens in college males and females were correlated with personality characteristics reflecting measures of emotionality, femininity, sensation-seeking, and sexual and social attitudes and behavior. First, a pilot sample of 25 college males was administered the Sensation-Seeking Scale (SSS) and the Eysenck Personality Inventory and two subscales, SSS, Disinhibition, and EPI, Neuroticism were significantly positively correlated with plasma androgens. Only androgens were assayed in the pilot sample. Then a new sample of 51 college males and 7 college females (not taking steroid contraceptives) was administered the California Psychological Inventory (CPI), Sensation-Seeking Scale, FIRO-B, EPI, Human Sex Questionnaire, Self-Rating Scale, Feminism Scale, and State Measure Sensation Seeking (SMSS). MMPI scale scores were derived from the CPI. Morning blood samples (10 ml) were drawn twice over a ten day period in both males and females; in females, blood samples coincided with follicular and luteal stages of menstrual cycle. Plasma was assayed for androgens by means of competitive protein binding and for estrogens by radioimmunoassay. The dependent variable hormone measurements included an "average" androgen and estrogen level, as well as their relative ratio (androgen/estrogen). Both simple and partial psychoendocrine correlations were computed, with partial correlations being simultaneously controlled for body weight, age, height, and recency since orgasm. Nine hypotheses were formulated dealing with measures of dominance, emotionality, depression, psychopathology, aggression, sensation seeking, sexuality, and psychological sex differences and related to circulating plasma levels of androgen and estrogen. In males, positive relationships were found between the sex hormones and state and trait anxiety, emotionality, sensation seeking (Disinhibition), and attitudes toward raising children. Negative relationships were found between the sex hormones and measures of social dominance, variety of heterosexual experience, and femininity. The androgen/estrogen ratio significantly correlated with measures of psychosocial orientation, gender identity, and feminism but no relationship was found between the sex hormone ratio and psychological sex differences. In the smaller female sample significant simple correlations were found with follicular and luteal androgens and estrogens and sensation seeking (Disinhibition), emotionality, sexuality, and psychopathology. Personality profiles are presented and describe the high androgen, high estrogen, and high androgen/estrogen college male and female. Theoretical issues focus upon the biological basis of the sensation seeking motive, the psychoendocrinology of reward and punishment, the hormonal basis of CNS cortical excitation-inhibition, and the neuroendocrine basis of schizophrenia.

929 STEROID CONCENTRATING CELLS IN THE BRAIN OF THE PARADISE FISH: AUTORADIOGRAPHIC LOCALIZATION. Roger E. Davis, Joan I. Morrell and Donald W. Pfaff[1]. The University of Michigan, Ann Arbor, MI.; [1]The Rockefeller Univ., N.Y.C.

The distribution of brain cells which concentrate sex steriods following administration of tritiated estradiol or testosterone was investigated. Ten adult male paradise fish, Macropodus operularis, from 4 to 6 g body weight, were castrated one week prior to i.p. administration of the labeled hormone. Five males received ^{3}H-estradiol (S.A. 91 Ci/mM), and five received ^{3}H-testosterone (S.A. 85 Ci/mM) in a dose of 4 µCi/g body weight. Two hours later the males were sacrificed in icewater. The brain was quickly removed and blocked for horizontal or transverse sectioning, and frozen onto a cryostat specimen holder with powdered dry ice. Serial, 6 µ sections were cut at -19°C. Autoradiograms were prepared under a safelight by mounting the unfixed, unembedded, frozen sections onto dry slides which were precoated with NTB-3 nuclear emulsion. The exposure was terminated at 6 or 9 months for selected brain sections. The developed slides were systematically scanned with the aid of a light microscope and then the locations of labeled cells were charted on detailed anatomical drawings of the sections. A cell was designated to be labeled when the concentration of reduced silver grains over a clearly visible cell body was 5 times the reduced silver grains over an adjacent cell-sized area of neuropil (background).

After 6 or 9 months exposure, the number of labeled brain cells was greatest in males which had received the estradiol but the distribution of labeled cells in the brain was similar for both hormones. The inferior lobe contained the largest number of labelled cells, specifically throughout the lateral tuberis nucleus, and in the recessus lateralis nucleus (nomenclature of Peter and Gill, 1975, J. Comp Neurol., 159, 69). Many hormone-concentrating cells were also found in the preoptic nucleus, and in specific, limited areas of the anterior pituitary. An area within the anterior tuberis nucleus and a subpallial area of the telencephalon, rostral to the anterior commissure also contained labelled cells.

930 SEROTONERGIC DRUGS MODULATE THE CORTICOSTEROID RESPONSE TO 5-HYDROXY-TRYPTOPHAN. Jerrold S. Meyer, Neil S. Buckholtz and William O. Boggan. Depts. of Psychiatry and Biochemistry, Med. Univ. of So. Carolina, Charleston, SC. 29401

Although the serotonin (5-HT) precursor 5-hydroxytryptophan (5-HTP) is known to stimulate pituitary-adrenal activity in a number of species, little evidence has been presented concerning a) whether 5-HT in fact mediates this response, and b) whether the presumed serotonergic receptors are located within the brain. We investigated these questions in adult female CF1 mice given L-5-HTP (25 or 50 mg/kg i.p.) and pretreated with one of a variety of drugs which influence serotonergic function. Control mice received the appropriate drug vehicles (usually saline) in each case. Mice were decapitated 1 hr. following 5-HTP or its vehicle and plasma corticosterone concentrations measured by competitive protein-binding assay.

Lilly 110140 (10 mg/kg, 1 hr. pretreatment), a drug which inhibits 5-HT reuptake, markedly potentiated the corticosteroid response to 5-HTP. The putative 5-HT receptor blockers cyproheptadine (7.5 mg/kg, 30 min. pretreatment) and methergoline (3 mg/kg, 1 hr. pretreatment) attenuated the response by 41% and 25% respectively. Treatment with parachlorophenylalanine methyl ester (375 mg/kg daily for 5 days), a drug which depletes endogenous 5-HT by inhibiting tryptophan hydroxylase, failed to alter the effect of 5-HTP. However, this is explained by the fact that 5-HTP administration bypasses the metabolic block, thus providing for the synthesis of new 5-HT. Finally, the corticosteroid response to 5-HTP was almost completely abolished by administering Ro 4-4602 (25 mg/kg, 30 min. pretreatment) or MK 486 (40 mg/kg, 30 min. pretreatment), drugs which, at these doses, block the conversion of 5-HTP to 5-HT (by inhibiting l-aromatic amino acid decarboxylase) in the periphery but not the brain.

Taken together, these results strongly suggest that there exist, in the mouse, serotonergic receptors which can activate the pituitary-adrenal system. However, in contrast to what has usually been assumed, these receptors appear to lie outside of the brain.

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931 EFFECT OF PEPTIDE HORMONES ON EXTRACELLULAR ELECTRICAL ACTIVITIES OF PREOPTIC-HYPOTHALAMIC NEURONS. Robert L. Moss, Martin J. Kelly* and Carol A. Dudley*. Dept. Physiol., Univ. of TX Hlth. Sci. Cntr., SW Med. Sch., Dallas, TX 75235.

The action of peptide hormones on preoptic (PO)-hypothalamic neurons was investigated in urethane anesthetized, ovariectomized female rats. Localization of the tested neurons was accomplished by antidromic stimulation, and/or reconstruction of the electrode tract, and in some cases confirmation by histological examination. Multi-barrelled glass micropipettes were used for extracellular recording and for luteinizing hormone-releasing hormone (LRH), LRH analog (an agonist) and thyrotropin releasing hormone (TRH) microelectrophoresis. To insure the integrity of the response, repetitive testing of the active agent and the microelectrophoresis of Na and Cl ions, as current controls, were routinely performed. In the first series of experiments four specific neuron types were recorded and tested with LRH. Type I represented PO-neurons whose axons terminated in the arcuate (ARC)-median eminence (ME) complex while Type II were PO-neurons whose axons projected to other neural sites. Type III represented ARC neurons whose axons terminated in the ME region while Type IV were ARC neurons whose axons projected to some other site. The percentage of neurons displaying excitation (+), no response (0) or inhibition (-) to the microelectrophoresis of LRH are presented in the table below.

TYPE OF NEURON	N	EXCITATION	NO RESPONSE	INHIBITION
I	63	27%	67%	6%
II	183	32%	52%	16%
III	31	30%	60%	10%
IV	168	46%	42%	12%

Nearly half of the Type IV neurons showed a reproducible excitation to LRH. The LRH-elicited response was virtually instantaneous and coterminous with the period of hormone application. However, Type I, II and III neurons were found to be mainly non-responsive to LRH. In order to test the specificity of the LRH-induced excitation, LRH, TRH, as well as an LRH analog (agonist) were microelectrophoresed on a variety of neurons located in the PO area, ARC nucleus, anterior hypothalamus, ventromedial nucleus, septal area, thalamus and cortex. Preliminary findings of the combined data for all neural sites showed that approximately half of the neurons tested with LRH [N=505; (+)37%, (0)48%, (-)15%] and TRH [N=131; (+)15%, (0)62%, (-)23%] displayed no response.However, of the neurons responding, more neurons were inhibited by TRH than excited, and more were excited by LRH than inhibited. On the other hand, nearly half of the neurons tested showed a marked excitation to the microelectrophoresis of the LRH analog (agonist) [N=112; (+)48%, (0)32%, (-)20%]. The majority of neurons (83%) that demonstrated an excitation to LRH also showed excitation to LRH analog, and either inhibition or no effect to TRH. The presence of neuropharmacologically active peptides in the brain raises the possibility of novel mechanisms for chemical information transfer. (Supported by NIH Grant NS10434)

932 INTERACTION OF OLFACTORY AND AMYGDALA DESTRUCTION WITH SEPTAL LESIONS: EFFECTS ON LORDOSIS BEHAVIOR. D. M. Nance, M. McGinnis* and R. A. Gorski. Dept. Anat. and Brain Res. Inst., Sch. Med., UCLA, Los Angeles, CA. 90024.

Septal lesions (SL) produce a marked increase in behavioral sensitivity to estrogen in female rats. Olfactory ablation (OA) is reported to facilitate lordosis behavior in rats, but whether this is due to an increased sensitivity to estrogen has not been examined. In the first experiment, adult female rats were ovariectomized, had their olfactory bulbs removed by aspiration or were given sham operations, and tested for lordosis behavior three weeks later. Animals were primed with 2 ug estradiol benzoate (EB) for 3 days and tested on the fourth day in a plexiglass arena with 2-3 sexually vigorous Long-Evans male rats (20 mount test). The test was replicated 3 weeks later (Test 2). A lordosis quotient (LQ) was computed for each animal as # lordosis responses/# mounts X 100.

	Test 1	Test 2	
OA	72.14	81.42	*
SHAM	13.12	16.25	

OA animals showed a significantly higher mean LQ than sham operated rats. Since OA increases behavioral sensitivity to EB as shown for SL, in Experiment 2 we directly compared the effects of OA and SL on behavioral sensitivity to EB. To assess whether the effects of an OA and SL were additive, additional rats were given both lesions (SL+OA). All animals were gonadectomized and tested as in Experiment 1, except that the animals were tested at 3 week intervals with 0.5, 1.0 and 2.0 ug doses of EB.

	Dose of EB X 3 Days			
	0.5 ug	1.0 ug	2.0 ug	
SL	35.55	70.00	92.22	*
SHAM	17.14	30.71	42.85	
OA	47.50	75.00	78.75	*
SL+OA	61.25	65.00	87.50	*

The dose-response curves for the OA and SL groups were identical and significantly above those of the sham operated controls. The SL+OA group was comparable to the OA and SL rats, indicating the effects of OA and SL are nonadditive. Thus, both OA and SL facilitate lordosis behavior by producing an increase in behavioral sensitivity to estrogen and may be mediated by a common neural mechanism. In the last experiment, spayed rats were given dual lesions of the septum and amygdala (AL), and the response to EB (2 ug X 3 days) compared to SL, AL and sham animals as above.

	Test 1	Test 2	
SL	77.00	83.00	*
SHAM	34.38	25.00	
AL	33.00	42.00	
SL+AL	25.00	43.00	

Only the SL rats showed an elevated LQ; the dual lesion (SL+AL) animals and AL rats were comparable to their sham controls. Thus an AL can attenuate the facilitatory effects of a SL on lordosis behavior of female rats. The fact that amygdala lesions, which alone do not alter lordosis behavior, block the facilitatory effect of SL may be explained in several ways. The balance between amygdala and septal modulation of a common behavioral system may determine hormone responsiveness; ie., the facilitation of the LQ following SL may be due to a greater influence of the amygdala. Or, SL may increase the LQ of female rats by producing a type of denervation hypersensitivity in the remaining estrogen-sensitive neural tissue, and the amygdala could be an important site of this process. The comparable facilitatory effect on lordosis of OA to SL indicates that these brain areas may exert a similar tonic inhibition on lordosis behavior. (Supported by USPHS Grant No. HD-01182.)

*Significantly different from SHAMS (analysis of variance).

933 MICROCHEMICAL ANALYSIS OF THE HYPOTHALAMIC LESION INDUCED BY MONOSODIUM L-GLUTAMATE (MSG) IN THE NEONATAL RAT: EVIDENCE FOR A DOPAMINERGIC ROLE IN NEUROENDOCRINE REGULATION. C.B. Nemeroff, R.J.Konkol*, J.B.Martin, G. Bissette*, P.Brazeau, G.R.Breese, L.D.Grant, A.J.Prange, Jr.* and J.S. Kizer*. The Neurobiology Program, Univ. North Carolina, Chapel Hill, N.C. 27514 and The Division of Neurology, McGill University, Montreal, Quebec, Canada.

Although it is generally agreed that catecholaminergic neurons within the hypothalamus are involved in central neuroendocrine regulation, controversy nevertheless exists concerning the relative importance of dopamine (DA) and norepinephrine (NE) in this regulation. Previous work from this and other laboratories has shown that treatment of newborn mice, rats and monkeys with monosodium l-glutamate (MSG) results in brain lesions in the adult, largely restricted to the arcuate nucleus of the hypothalamus and the retina. As adults, newborn rats so treated are stunted in stature and obese and have smaller uteri, gonads and pituitaries than sex-matched controls. Fluorescent histochemical studies of the arcuate nucleus indicate a nearly complete loss of DA perikarya, whereas other catecholaminergic systems within the CNS, both hypothalamic and extrahypothalamic, appear intact (Neuroscience Abs. 1,1975,434). Thus adult rats bearing the MSG-induced arcuate nucleus lesion can serve as a useful model to study the importance of the tuberoinfundibular DA system in neuroendocrine regulation. Litters obtained from time-pregnant Holtzman rats were treated either with MSG (4 mg/g BW) or vehicle (10% NaCl) on alternate days for the first ten days of life. At 18 weeks of age animals were decapitated, their brains rapidly removed and frozen on dry ice. Specific hypothalamic and brain nuclei were removed by the microdissection technique of Palkovits (Brain Res. 59,1973,449). Thyrotropin-releasing hormone (TRH) and luteinizing hormone-releasing hormone (LHRH) were measured in the ventrobasal hypothalamus by radioimmunoassay (RIA) procedures as previously described (Endocrinol. 98,1976,449). DA, NE and serotonin (5-HT) were measured in discrete nuclei by radioenzymatic procedures (J.Neurochem. 21,1973,63; J.Pharmacol. Exp. Ther. 186,1973,508). Trunk blood was obtained from each animal after decapitation and serum levels of growth hormone (GH), thyrotropin (TSH) and prolactin (PRL) were measured by RIA (NIAMDD Rat Pituitary Reagents). Neonatal treatment with MSG resulted in stunted, obese adults with markedly reduced gonadal and uterine weights. Although serum TSH and PRL were unchanged, serum GH levels were markedly reduced in the MSG-treated females (12.1 ± 4 ng/ml) when compared with sex-matched controls (52.0 ± 17 ng/ml; $p<0.05$). Although GH levels were lower in the MSG-treated male rats, the differences just failed to attain statistical significance. Microchemical analysis revealed no difference in immunoreactive LHRH or TRH within the medial basal hypothalamus in either the MSG-treated males or females when compared to sex-matched controls. In addition concentrations of 5-HT and NE in the median eminence, arcuate nucleus, medial forebrain bundle, suprachiasmatic nucleus and dorsomedial nucleus were unchanged after neonatal MSG treatment. DA levels in the dorsomedial, substantia nigra, striatum, and paraventricular nuclei were also normal. There was, however, a significant decrease in DA levels in the arcuate nucleus (↓50%) and median eminence (↓62%). Thus the disruption of endocrine homeostasis in the MSG animals is associated with normal hypothalamic levels of LHRH, TRH, NE and 5-HT, whereas arcuate and median eminence DA is substantially reduced. This study provides further evidence to support the hypothesis of a predominant role for the tuberoinfundibular DA system in endocrine feedback regulation and suggests that the role of 5-HT and NE in this regulation may be of minor importance in the mediobasal hypothalamus.

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934 EFFECT OF NEONATAL ADMINISTRATION OF MONOSODIUM GLUTAMATE ON REPRODUCTION IN THE ADULT MALE AND FEMALE MOUSE. William J. Pizzi, June E. Barnhart* and Don J. Fanslow*. Neuropsychology Lab, Northeastern Ill. Univ., Chicago, Ill., 60625.

Monosodium glutamate (MSG) has been shown to produce lesions in the brains of various mammals. This damage occurs primarily in structures contiguous with ventricular cerebral spinal fluid, and is best demonstrated in the arcuate nucleus of the hypothalamus. Accompanying this CNS damage there are reports of several somatic and behavioral dysfunctions including stunted skeletal growth, obesity, abnormal activity levels, sterility in female mice and learning deficits. This study will report on the reproductive capacity of both female and male mice treated with MSG during the neonatal period. The data from these reproduction studies along with the weights of various endocrine glands indicates that neonatal administration of MSG results in an impairment of the hypothalamic-hypophysial regulation of reproduction. The pattern of endocrine dysfunction may also serve to explain several of the other somatic and behavioral impairments seen in MSG-treated organisms.

The purpose of the present study was to quantify MSG-induced reproductive deficits in both female and male mice. To this end, MSG or a control vehicle was administered subcutaneously to animals (N=50) from days 2-11 of life according to a dose schedule prescribed by Potts et al (Amer. J. Ophthalmol., 50: 900, 1960). In the first study 5 mating environments were set up, each consisting of 2 MSG-treated females, 2 control females, and 1 normal male. The mating period lasted for 60 days, starting when the animals were 200 days of age. MSG-treated females failed to become pregnant as often as control littermates (C=9 of 10 vs. E=3 of 10, $p < 0.009$) and produced significantly smaller litters (C=10.66 vs. E=4.00, $p < 0.02$). MSG-treated males and controls were autopsied between 295 and 302 days of age. MSG-treated males exhibited significantly greater body weights, and significantly lower weights of pituitary, thyroid, and testes. No weight difference was found in adrenal glands.

The reduction in weight of those endocrine glands necessary for reproductive function in the male led to a second study designed to test for reproductive dysfunction in both female and male mice treated with MSG. The procedure was the same as in the first study except that animals were mated at 100 days of age, and the mating period lasted 30 days. Six mating environments were set up to test reproductive ability of MSG-treated and control females, and 11 environments consisting of 1 MSG-treated male and 1 control female were set up to test the fertility of MSG-treated males. Results of the second study showed that in female mice, neonatal administration of MSG resulted in a significantly delayed sexual development, indicated by late vaginal canalization. Examination of daily vaginal smears indicated that females treated with MSG as neonates exhibited significantly longer estrus cycles as adults, spent significantly longer time in metestrus, and significantly less time in proestrus. This second study on female reproduction replicates the earlier study and shows a decreased number of pregnancies (C=12 of 12 vs. E=5 of 12, $p < 0.0002$) and reduced litter size (C=10.08 vs. E=4.80, $p < 0.0005$) in the MSG-treated mice. At 191 days of age autopsy of the female endocrine glands showed the same pattern as that of the males with pituitaries, thyroids and ovaries showing significantly reduced weights. MSG-treated males also failed to impregnate control females as readily as did control males (C=12 of 12 vs. E=6 of 11, $p < 0.01$). When these MSG-treated males were successful in impregnating control females there were no untoward effects as seen by litter size and birth weights. MSG-treated males, autopsied at 171 days of age, again evidenced significantly increased body weights and significantly reduced pituitary, thyroid, and testes weights.

935 ULTRASTRUCTURAL LOCALIZATION OF RADIOLABELED L-DOPA AND DOPAMINE IN THE ENDOCRINE HYPOTHALAMUS OF THE RAT. David E. Scott, Gerda Krobisch-Dudley* and Nadja Kutyreff*. Dept. of Anat., Univ. of Roch., Roch., NY 14642.

Male rats (250-300 gm) were anesthetized and infused via the right cerebral ventricle with tritiated L-dopa (15 c/mM) or tritiated dopamine (20 c/mM). Controls received saline only. Animals were killed 10, 20, 40 and 60 min. post-infusion and prepared for routine light and transmission electron microscopic autoradiography. Significant uptake and labeling as assessed with double blind analysis of variance was noted for the cytoplasmic matrix of neurons in the dorsal arcuate-periventricular region 10 and 20 min. following infusion of radiolabeled L-dopa. A similar highly restrictive pattern of selective uptake was also observed for radiolabeled dopamine in alternate animals. Other regions of the hypothalamus and adjacent thalamus were negative. However neurons in the parenchyma of the area postrema demonstrated light but significant labeling. Tanycytes of median eminence also demonstrated silver grains sequestered along their linear axis as they course between the cerebral ventricle and the hypophyseal portal bed.

Selective uptake and sequestration of either tritiated L-dopa, a catecholamine precursor and/or dopamine itself argues in favor of a catecholaminergic function for such neuronal populations in the arcuate nucleus and area postrema. This phenomenon may represent the structural correlate of a monoaminergic recapture mechanism by the perikarya of such neurons. An alternative explanation for uptake of radiolabeled dopa or dopamine by highly restricted neuronal perikarya may center about an autoregulatory short-loop feedback mechanism. In such a paradigm sub-populations of the heterogenous arcuate-periventricular pool or other regions such as the area postrema may be sensitive to the very products that they or adjacent neuronal pools synthesize. These sub-populations of neurons may serve as receptors which in turn may facilitate or inhibit adjacent neurons which may be active in the synthesis of releasing hormones or biogenic amines. The uptake and sequestration of ^{3}H-L-dopa, ^{3}H-dopamine or their metabolites by tanycytes of the median eminence is not a new finding but simply reinforces the ideatum that small physiologically active molecules are readily taken up by this cellular compartment. These observations are consistent with an absorptive role for this type of metaglial cell. However, whether this apparent transport capacity has any neuroendocrine impact upon the adenohypophyseal metabolism and the peripheral endocrine milieu awaits further elucidation.

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936 IMMUNOCYTOCHEMICAL LOCALIZATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH) IN THE HYPOTHALAMUS OF THE RHESUS MONKEY. A. J. Silverman, J. Antunes*, M. Ferin*, and E. A. Zimmerman. Depts. Anat., Neurosurgery, Neurology and Physiol., College of P & S, Columbia Univ., N.Y., N.Y. 10032

The distribution of LH-RH was investigated in hypothalami of male and female rhesus monkeys by light microscopic immunocytochemistry. Brains were fixed in Bouin's solution, embedded in paraffin and 6 μm sections cut and stained by sequential application of antiserum to LH-RH (provided by S. Sorrentino, Jr.), sheep anti-rabbit globulin serum, the peroxidase-anti-peroxidase complex (provided by L.A. Sternberger) and 3,3'-diaminobenzidine-H_2O_2. The absorption of antiserum to LH-RH with synthetic LH-RH prior to its application to tissue sections reduced or eliminated reaction products.

LH-RH positive cell bodies were found scattered throughout the entire hypothalamus but were most frequently observed in the following locations: lamina terminalis; diagonal band of Broca; pericommissural region of the anterior hypothalamus; medial basal hypothalamus, including the infundibular nucleus and the area lateral to the infundibular nucleus at the level of the median eminence; and premammillary body. No magnocellular neurons were immunoreactive.

LH-RH containing axons were distributed throughout the hypothalamic-septal region. Axons were observed in the lamina terminalis and diagonal band of Broca and entered the organum vasculosum of the lamina terminalis (OVLT) where some of them terminated on the capillaries. These terminals originated from the perikarya in the pericommissural region. Other axons traveled around the anterior commissure and a few ran in a ventromedial direction. Other fibers were observed in the medial basal hypothalamus and projected to the median eminence (ME). Within the ME itself, LH-RH fibers formed a fine, diffuse network throughout the external zone; many axons were traced along the infundibular stalk and into the posterior pituitary where they terminated on the large capillaries bordering the posterior pituitary and the pars intermedia. Another group of axons were present in the mammillary body.

In the rhesus monkey LH-RH is distributed as a diffuse network of neurons throughout the hypothalamus and fibers containing the peptide establish neural-heamal contacts in three sites: OVLT, ME and posterior pituitary. The perikarya of origin of these nerve terminals has not been firmly established.

937 CHANGES IN A POPULATION OF RAT BASOMEDIAL HYPOTHALAMIC NEURONS CORRELATED WITH THE ENDOCRINE STATE OF THE ANIMAL. Gary K. Steinberg* and Terry L. Powley.* (SPON: K. L. Chow), Dept. of Anat., Sch. Med., Stanford Univ., Stanford, CA. 94305 and Dept. of Psych., Yale Univ., New Haven, Conn. 06520.

Using light microscopic techniques and Nissl staining procedures, we have identified a population of basomedial hypothalamic neurons that appears anatomically and functionally distinct. The staining affinity of these neurons changes with the endocrine status of the animal, and the cells are most easily recognizable when they stain with their characteristic shape (fusiform), intensity (very dark cytoplasm and nucleoplasm), and orientation (all cells oriented along the same axis). This population of fusiform neurons is not confined to any previously defined hypothalamic nucleus; rather, it forms a coherent cell field that condenses at the ventrolateral pole of the ventromedial nucleus of the hypothalamus. The distribution of this cell field is constant among all animals examined, extending from the middle of the ventromedial nucleus through the posterior end of the ventral premamillary nucleus, from the ventral surface of the brain up to the fornix, and occupying the middle half of the hypothalamus in this region.

Several studies we have performed provide evidence that this anatomically defined population of neurons is also functionally distinct. By sacrificing female rats on different days of the 4-day estrous cycle, we demonstrated that the density of fusiform neurons varies predictably during the estrous cycle. On the morning of diestrus-2 and proestrous, 50-60% of the total number of neurons in the hypothalamic distribution sampled exhibit the fusiform characteristics, while on the morning of estrus only about 20% of the neurons are fusiform, and on the morning of diestrus-1 about 30%. Hypophysectomy of both male and female rats causes a dramatic increase in the numbers of fusiform neurons throughout their distribution. Examination of brains from a strain of genetically obese rats (Zucker "fatty") demonstrated that the mutant rats have very few fusiforms, and interestingly, hypophysectomy of the genetically obese rats causes the sudden appearance of large numbers of fusiform, dark-staining neurons.

The explanation for why these basomedial hypothalamic neurons appear as dark-staining fusiform or light-staining round is unknown, but our working hypothesis is that the shape and staining characteristics of the neuron may be a reflection of the cell's activity. The distribution of these hypothalamic fusiform neurons corresponds in part with areas of the rat brain which have been implicated in various neuroendocrine and autonomic functions, as shown by ^{3}H-estradiol mapping, hormonal implants, lesions, localization of hypothalamic releasing factors, electrical stimulation, and electrophysiological recording. Presently, we are using several anatomical and physiological techniques to elucidate further the functional importance of this hypothalamic fusiform neuron population. (Supported in part by a MSTP Grant from the NIH GM01922).

938 BEHAVIORAL AND NEUROENDOCRINE EVALUATION OF TARDIVE DYSKINESIA.
CT Tamminga,* RC Smith, G Pandy, L Frohman,* JM Davis. (SPON: JM Davis). Illinois State Psychiatric Institute, Michael Reese Hospital, Depts. of Psychiatry and Medicine, University of Chicago.

As Tardive Dyskinesia is emerging as an important and persistent long term side effect of neuroleptic treatment, hypotheses of its pathophysiologic mechanisms are being explored. Pharmacologic data in evaluating stereotyped behavior in animals (Psychopharm. Comm. 1:272) and movement disorders in humans (J. Neurol. Sci. 14:189) have indicated that the involuntary, choreic movements intensify with some dopamine (DA) agonists and remit with DA antagonists. A chemical denervation hypersensitivity of the nigrostriatal DA system is being explored as the underlying mechanism of tardive dyskinesia. We have used pharmacologic probes in a tardive dyskinesia population (n=10) to evaluate that hypothesis both from a clinical response and from a neuroendocrine perspective. We have found that IV D-amphetamine, 15-30 mg over 20 minutes, intensified Tardive Dyskinesia symptoms 10-50%. However, apomorphine a direct acting DA agonist in acute increasing doses from 0.75 mg to 6.0 mg did not intensify but tended to decrease the tardive dyskinesia symptoms. Butaperazine as expected decreased the movements appreciably. When examining neuroendocrine parameters in a tardive dyskinesia population based on a dopamine hypersensitivity theory, one might expect to see hyperactivity of the pituitary hormones modulated by DA. We have examined the Growth Hormone (GH) and Prolactin systems with a number of pharmacologic probes including an apomorphine test and the standard CPZ challenge. Our results show that after a 0.75 mg subcutaneous dose of apomorphine the peak GH response (X ± SE ng/ml) was 18.0 ± 3.1 in normal controls; 12.3 ± 3.7 in chronic schizophrenics and 12.0 ± 2.4 in patients with tardive dyskinesia; these means are not statistically different. Basal prolactin levels were comparable in the three groups, and there were similar patterns of response to apomorphine in the groups. In preliminary results there were no observed differences in basal prolactin or in patterns of suppression. We will also present GH and Prolactin response to a CPZ challenge in the three populations. Based on the clinical response of the movement disorder to DA agonist drugs and on neuroendocrine description of DA response we find little to support the theory of DA hypersensitivity in tardive dyskinesia. Certainly the hypersensitivity could be localized to motor tracts and not involve either the thought disorder sites or the tuberoinfundibular tract. Some of the seemingly paradoxical apomorphine effects of decreasing tardive dyskinesia symptoms may be related to presynaptic rather than post synaptic actions of the drug on central dopamine neurons. (Supported by FFRP 74-592 and FFRP 73-578)

939 NEUROENDOCRINE CONCOMITANTS OF INTRACRANIAL SELF-STIMULATION: EFFECTS ON GROWTH HORMONE (GH), PROLACTIN (PRL) AND CORTICOSTERONE (CS). Leon C. Terry*, Judy Audet*, Paul Brazeau* and Joseph B. Martin. Div. Neurology, Dept. Medicine, The Montreal General Hospital and McGill University, Montreal, Quebec, Canada.

Intracranial self-stimulation (SS) is reported to cause hormone release from the adrenal medulla and cortex. However, there is little information on the effects of SS on hypothalamic-pituitary regulation. In the present studies, plasma GH, PRL and CS were determined in control, SS and passively-stimulated (PS) rats.

Monopolar electrodes were implanted in the lateral hypothalamic-medial forebrain area of male Sprague-Dawley rats. Electrode placements were verified histologically. An indwelling, intra-atrial catheter was inserted to allow repeated blood sampling without disturbance to the animal. Rats were housed individually in isolation cages and conditioned to self-stimulate at a constant rate. Rates of SS varied from 2111 to 6467 bar presses (BP) per hour in six different rats. Blood samples were removed every 15 minutes over 5-6 hour periods for determination of GH, PRL and CS. Each animal was sampled on 4 to 6 different days. The first day was without stimulation, the second and third were SS each alternate hour with subsequent reversal of the pattern, and the fourth was passive stimulation (PS) using identical stimulus parameters and frequency. In most animals, a fifth day of baseline sampling was done.

Normal episodic bursts of GH were suppressed during periods of SS with a surge occurring immediately after cessation of SS. Passive stimulation had the same effect. Higher rates of SS altered the GH pattern more than lower rates. Mean peak GH levels during SS were 80.0±20.1 compared to 273.1±18.8 ng./ml. for non-SS periods. PRL rose immediately and remained elevated during periods of SS. Mean peak PRL levels during SS were 122.2 ±38.3 compared to 14.6±2.5 ng./ml. in non-SS intervals. There was no apparent difference between SS and PS. Peak PRL levels were 3 to 4 times higher during the first hour of SS or PS compared to the second and third hours with similar BP rates. Corticosterone followed a similar pattern.

These results suggest that pituitary hormone changes accompanying SS are similar to those known to occur with stress and indicate that SS causes nonspecific activation of the hypothalamic-pituitary axis.

(Supported by the Medical Research Council of Canada).

940 DNA AFFINITY SEPARATES ANDROGEN FROM ESTROGEN BINDING ACTIVITY: STUDY OF RESIDUAL ANDROGEN BINDING MACROMOLECULES IN Tfm MUTANT MOUSE BRAIN.

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An earlier study (Fox, PNAS 72: 4303, 1975) demonstrated two sex steroid binding activities in hypothalamus-preoptic area of the mouse. One bound estradiol with high affinity and specificity and is hereafter called E-R (presumed estradiol "receptor"). The second activity bound either testosterone or dihydrotestosterone with similar high affinities, but was easily blocked by estradiol. This activity, referred to here as A-R (presumed androgen "receptor") was deficient in the androgen-insensitive mutant, testicular feminization (Tfm). To allow quantitative comparisons of E-R and A-R steroid affinities and possible interactions of E-R with A-R, we have devised a method for separating these two entities using DNA-cellulose affinity chromatography.

At 50 mM NaCl, both A-R and E-R bind to DNA-cellulose, but not to cellulose alone. Both can be eluted at 210 mM NaCl, but to achieve 90% separation, A-R can be eluted at 130 mM NaCl, without eluting E-R. The procedure used was as follows: extracts prepared in 50 mM NaCl were labeled with 10 nM H^3-steroid (either estradiol, testosterone, or dihydrotestosterone), then passed over Sephadex G-25 to separate macromolecular-bound from unbound hormone. The macromolecular portion was applied to DNA-cellulose in 50 mM NaCl, and eluted with 20 mM steps of NaCl. A-R eluted at 110-130 mM NaCl, while E-R eluted at 190-210 mM NaCl. The same quantitative yield was obtained with single steps at 130 mM and 210 mM NaCl. In this case, as much as 93% of the DNA-bound A-R eluted at 130 mM, while only 2% of the E-R did. By sucrose gradient sedimentation both high and low affinity steroid binding are distinguished. Virtually 100% of the A-R adheres to DNA-cellulose, while none of the low-affinity hormone-binding activity adheres. This contrasts with estradiol binding from hypothalamus-preoptic area, of which only 50% of E-R adheres to DNA-cellulose, while again none of the low-affinity activity adheres.

DNA-cellulose chromatography allows further characterization of A-R from mouse hypothalamus-preoptic area as well as examination of the residual A-R in Tfm. Earlier work had indicated that hormone affinity and sedimentation behavior of Tfm and normal A-R were similar; however, these apparently normal parameters do not rule out other abnormalities in the protein. Chromatography on DNA-cellulose indicates that Tfm A-R can adhere at 50 mM NaCl and elute at 210 mM NaCl, as can normal A-R. In Tfm the high affinity binding (A-R) is reduced to 10% of normal levels; low affinity binding is unchanged. In Tfm as in normal, all of the A-R adheres to DNA-cellulose, while none of the low-affinity hormone-binding activity adheres.

Thus far, no difference between residual Tfm A-R and normal A-R has been demonstrated for hormone binding, sedimentation or DNA adherence. The evidence therefore supports the suggestion that the Tfm locus is a mutation that affects the level of A-R. Because the method described above yields quantitative partial purification of A-R, we can now collect larger quantities of Tfm A-R, allowing further comparative studies with normal. We can also use DNA binding to study the regulation of A-R levels.

These studies provide the first demonstration and partial characterization of DNA binding by A-R from brain.

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941 UNIT ACTIVITY OF THE NEUROHYPOPHYSIS OF NORMAL AND MESENCEPHALIC LESIONED RATS AND ITS MORPHOLOGICAL BASIS. Guillermo A. Zeballos*, Janett Trubatch and Alan B. Rothballer, Depts. of Neurosurgery and Physiology, New York Medical College, New York, NY 10029.

The unit activity of the neurohypophysis of anesthetized normal rats and unanesthetized rats with a mesencephalic section has been studied. Electron-microscopical studies were done to understand better the complex responses obtained from the gland following hypertonic stimulation[1]. The pituitary gland was exposed through the basisphenoid, unit activity recorded with tungsten microelectrodes (7-14 megohms) and monitored through differential preamplification and oscilloscopes. Activity was analyzed by computer-generated frequency histograms. Stimulation was done by injecting hypertonic solutions (1 M NaCl) into the internal carotid artery[2]. Mesencephalic (M) rats prepared by transection of the brainstem with a spatula from the rostral border of the superior colliculi to the rostral border of the pons. Recordings were made from normal (N) rats under anesthesia (Dial-urethane), and from M rats without anesthesia since they lack intact pain afferents. In normal rats, hypertonic injections gave three types of responses: a) Repetitive bursts (2 to 3), with a significant decrease in the rate of discharge immediately after the first burst following injection; b) Prolonged increase in the rate of discharge (4 to 10 minutes); c) Prolonged decrease in the rate of discharge (4 or more minutes). In the same locus, repetitive injections of hypertonic solutions generally changed the intensity of the response but not the pattern. In M rats, following hypertonic stimulation only burst type (1 to 2) responses were obtained and no significant decrease in the rate of discharge below control levels has been observed. Administration of a short-lasting anesthetic (tribromo-ethanol) caused a significant decrease in the basal rate of discharge in M rats.

The ultrastructure of the gland provides evidence that the different types of response obtained in the normal animal can be related to the location of the tip of the microelectrode within the neurohypophysis. In the neural lobe the axons have diameters of less than 0.5 microns and often run in bundles. This may explain the difficulty in isolating a single unit since the tip of the electrode itself is 2-3 microns. In addition, close to the perivascular space axon endings contain both neurosecretory granules and microvesicles or only the microvesicles, whereas away from the blood vessels the swellings contain many neurosecretory vesicles but few if any microvesicles. This regional difference may be reflected by different patterns of electrical activity.

M rats have not shown a decrease in the rate of discharge following strong stimulation of the system, as if an inhibitory source located behind the section has been eliminated. This interpretation is supported by the fact that previous works suggest the existence of a bulbohypothalamic inhibitory pathway, and by the clinical observation that patients suffering a lesion of the brainstem secondary to acute trauma often show the syndrome of inappropriate antidiuretic hormone release[3].

(Supported in part by USPHS NIH Grant NB #06624 and NSF Grant BMS 75 01611.)

References: [1]Rothballer, A. B., C. A. Munoz and G. A. Zeballos. Fed. Proc., 35: 690, 1976. [2]Zeballos, G. A., J. Thornborough and A. B. Rothballer. Neuroendocrinology 18: 104, 1975. [3]Rothballer, A. B. Res. Publ. Assoc. Neuro. Ment. Dis. 43: 86, 1966.

942 PROLACTIN LEVELS IN MALE RATS WITH MIDBRAIN RAPHE LESIONS. Juan P. Advis*, Greg Mueller*, Joseph Meites*, and James L. Bennett. Dept. of Physiol., and Pharmacol., Mich. St. Univ., E. Lansing, MI. 48824.

Previous reports have indicated that 5-hydroxytryptamine (5-HT) could be involved in regulating prolactin release e.g., d-lysergic acid diethylamide (PSEBM 137: 1242, 1971), parachlorophenylalanine (PCPA), or 5,7-dihydroxytryptamine (PSEBM 151: 512, 1976) when administered to rats would reduce basal levels of prolactin. Recently, it has been demonstrated that 5-HT turnover in the hypothalmus was increased after 15 minutes of restraint stress and at the same time prolactin levels were increased while thyroid-stimulating hormone levels were decreased (Life Sci.18: 715, 1976). It has been demonstrated that destruction of serotonergic cell bodies in the midbrain raphe nuclei will result in a degeneration of serotonergic fibers and terminals (Br. Res. 44: 165, 1972). To further investigate the relationship between 5-HT and prolactin we decided to study (a) the effect of raphe lesions on prolactin serum levels in male rats, and (b) study the effect of PCPA on stress induced increse in prolactin. One week after lesions were made we observed a 50% reduction in both 5-HT and Prolactin levels. Four days after a single dose of PCPA (400 mg/kg) we observed a 50% blockage of the stress induced increase in prolactin levels. Our observations indicate that the serotonergic neurons located in the midbrain raphe nuclei appear to be involved in regulating prolactin levels in male rats. (Research supported by NIH grant AM 04784).

943 AMYGDALOID LESIONS ABOLISH CORTICOSTERONE RHYTHMICITY IN THE RAT. J. P. Allen, Univ. of TX. Health Sci. Centr. and Audie Murphy VAH, and C. F. Rowlands, Southwest Fndn. for Res. and Ed., San Antonio, TX 78284.

Various extrahypothalamic central nervous system elements, including the amygdala, are involved in the control of both tonic and stress-induced ACTH and corticosterone secretion in the rat. Because the amygdala is essential for the hypersecretion of ACTH following adrenalectomy, we decided to test the hypothesis that the amygdala modulates circadian rhythmicity of corticosterone. This was accomplished by measuring diurnal plasma corticosterone levels after unilateral or bilateral discrete lesions were placed in the ventral amygdalo-fugal pathway or stria terminalis of 200 gram male Sprague Dawley rats. Lesions were made in these two principal amygdalo-hypothalamic pathways using a stereotactically guided knife or radio frequency lesion generator. These data were then compared to those obtained in sham-lesion control groups. One week after neurosurgery jugular vein blood was obtained at 800 and 1700 hours and plasma corticosterone concentrations measured by a sensitive and specific radioimmunoassay. A significant diurnal rhythm of plasma corticosterone concentration was present in sham lesioned controls ($P<0.01$), and in those with unilateral ventral amygdalo-fugal pathway lesions ($P<0.02$) or bilateral stria terminalis lesions ($P<0.01$). However, the diurnal rhythmicity of plasma corticosterone was abolished in animals with bilateral ventral amygdalo-fugal pathway lesions with morning plasma corticosterone levels not significantly different from the afternoon levels. Histologic verification of the lesions was performed.

These data suggest that the amygdaloid complexes of the rat are involved in the control of circadian rhythmicity of corticosterone and may function as a glucocorticoid rhythm modulator in the physiologic control of corticosterone secretion.

944 PARTICIPATION OF SEROTONIN NEURONS IN THE PHYSIOLOGIC SECRETION OF RAT GROWTH HORMONE. M. A. Arnold* and J. D. Fernstrom. Laboratory of Brain and Metabolism, Massachusetts Institute of Technology, Cambridge, MA. 02139.

The temporal pattern of growth hormone (GH) secretion was examined in the gentled male Wistar rat. Animals that had been adapted to our animal quarters (light period from 0800 to 2000 h) for one week were killed at half-hourly intervals between 1900 and 2200 h. Serum GH levels rose markedly just after the onset of the dark period (i.e., at 2000 and 2030 h). Pretreatment with the serotonin antagonist cyproheptadine (10 mg/kg, i.p.) at 1900 h blocked this nocturnal GH surge:

	SERUM GH (ng/ml)	
TIME OF DAY	Vehicle	Cyproheptadine
1900	19 ± 8	
2000	80 ± 27	20 ± 5*
2030	59 ± 12	31 ± 8*

*P<.05, Differs from controls (n=10); X ± SEM.

These data provide further evidence that serotonin neurons are involved in the mechanisms normally controlling the secretion of GH in the rat.

(Supported by a grant from the National Science Foundation)

945 EFFECTS OF CORTICOSTERONE AND 5α-DIHYDRO-CORTICOSTERONE ON BRAINSTEM SCIATIC EVOKED POTENTIALS. H. Barbas*, I. Kraulis* and B. Dubrovsky. (SPON: C. de Montigny), Neurophysiology Lab., Allan Memorial Institute, McGill University, Montreal, Quebec. In previous studies (Neurosc. Abs. 687, 1975), we have shown that DOC and its ring A reduced metabolite, 5α-dihydro-DOC, cause a decrease in sciatic evoked potentials in the pontine reticular formation (Pont. RF) of the adrenalectomized rat while corticosterone (compound B) causes an increase. We now wish to report that, contrary to its parent compound B, intraperitoneally administered 5α-dihydro-B (0.75 mg/300 g) also causes a decrease (19-38% below baseline amplitude; onset lag: 10-16 min; full recovery: 16-21 min) in sciatic evoked potentials in the Pont. RF and that this effect can be reversed (latency for full recovery 4 min) by a subsequent injection of an equivalent dose of compound B, indicating that a CNS excitatory steroid may counteract the central effects of a CNS depressant steroid. That the observed effects of adrenal steroids on CNS excitability need not be related to their neuroendocrine feedback mechanisms on pituitary-adrenal function is indicated by the fact that, at a dose of 1 mg/100 g injected subcutaneously, both B and DOC reduced stress levels of serum corticosterone from 21.4±2.3 μg per 100 ml to 6.4±0.7 and 6.4±2.0 μg., respectively, while 5α-dihydro-DOC (18.9±1.9) and 5α-dihydro-B (18.5±1.8) had no effect.

946 EFFECTS OF ESTROGEN AND 6-OHDA ON RECEPTIVE FIELDS OF FACIAL MECHANORECEPTOR NEURONS. D.A. Bereiter* and D.J. Barker* (SPON: C. L. Prosser). University of Illinois, Urbana, IL. 61801.

Previous studies demonstrated that receptive field areas (RFA) of individual mechanoreceptor neurons innervating the face in gonadectomized female rats were significantly larger following 2, 5 or 10 days of estradiol benzoate (EB) treatment (20 µg/day). RFA enlargement also occurred in normal cycling estrus females when compared to females in diestrus. The present experiments were designed to study (a) whether EB affects mechanoreceptor types differentially, and (b) possible mechanisms underlying the RFA enlargement. Threshold RFA of well-isolated single trigeminal ganglion neurons were determined (Von Frey technique) in acute recording experiments, and transferred to photographs for planimetric measurement. The results were as follows: (1) EB treatment for 5 or 10 days caused a preferential increase in RFA for rapidly adapting neurons. (2) There was no consistent change in mechanoreceptor force thresholds after EB treatment, suggesting no change in neuronal sensitivity to vertical forces. Possible changes in lateral distensibility were not measured however. (3) An acute epidermal hyperplasia (38.7%) after 10 days of EB treatment suggests that changes in skin mechanical properties may contribute to enlarged RFA. Epidermal hyperplasia is known to be correlated with sprouting of nerve terminals induced by chemical and UV treatment of skin. Although our results did not demonstrate nerve terminal growth (due to technical difficulties in staining hairy skin), neuronal sprouting cannot be ruled out as a possible mechanism. (4) Depletion of skin norepinephrine by injection of 6-hydroxydopamine produced RFA enlargements similar in magnitude to the EB induced increase, suggesting the possible involvement of norepinephrine in this phenomenon. Unlike EB treatment however, 6-OHDA significantly lowered force thresholds for rapidly adapting hair receptors.

947 RELATION OF PROGONADAL EFFECTS OF MELATONIN TO SCOTOREFRACTORINESS IN THE HAMSTER. Eric L. Bittman (SPON: Irving Zucker). Dept. of Psychology, University of California, Berkeley, California 94720.

Exposure to short photoperiods induces testicular regression in hamsters within 2 months. Recrudescence of the gonads may be induced by long days and will also occur spontaneously at a slower rate with continued exposure to short days. In either case recrudescence is followed by unresponsiveness to short photoperiods (scotorefractoriness) (Reiter, Anat. Rec. 173:365). Implantation of pellets containing melatonin (M), a pineal constituent, prevent short photoperiod induced testicular regression (Endocrinology, 96:206; Science, 190:280). This study tested Reiters suggestion that M's protective effect on the gonads might be due to induction of scotorefractoriness. Hamsters were implanted with empty or M-filled capsules of Silastic or beeswax upon transfer from long (LD 14:10) to short (LD 6:18 or LD 2:22) photoperiods. Beeswax pellets were replaced weekly. Laparotomy 8 to 10 weeks later confirmed the progonadal effects of M; unlike control animals, hamsters with M implants maintained large and functional gonads. M treatment was terminated at this time. The testes of experimental hamsters left in short photoperiods regressed and recrudesced with a time course identical to that of animals that had never been protected by M. Other animals previously protected with M were transferred from short to long days for 10 weeks. Upon return to the short photoperiod their testes regressed. These results suggest that the mechanism by which M protects the gonads from short-photoperiod induced regression does not involve rendering hamsters scotorefractory and that exposure to M prevents induction of scotorefractoriness by short days. The data are also consistent with the hypothesis that gonadal regression is necessary for the induction of scotorefractoriness.

948 EFFECT OF CORTICOSTERONE REPLACEMENT ON SPONTANEOUS ACTIVITY IN RATS WITH VMH LESIONS. William P. Brittain and Charles D. Lamade*. Department of Psychology, Lycoming College, Williamsport, Pa. 17701

It has been reliably demonstrated that VMH lesions (Hetherington and Ranson,1942;Sclafani,1972;Brittain,1973) as well as adrenalectomy (Fuller Chambers,and Fuller,1956;Leshner,1971) result in a marked reduction in spontaneous wheel running in rats. Other studies (Remley,Brittain,and Seago,1970;Brittain,1973) have shown that VMH lesions result in atrophy of the adrenal cortex. Leshner (1971) demonstrated that hypoactivity resulting from adrenalectomy could be returned to normal level with corticosterone replacement. The current study investigated some possible relationships between VMH-induced hypoactivity and corticosterone availability.

Six male rats were given bilateral VMH lesions (VMH),six rats were given bilateral adrenalectomies (Adx),and six Ss were sham operated as controls. Pre-surgery activity levels for the three groups were not different. Post-surgery activity measures showed both Adx and VMH groups to be significantly lower than controls. On the eighth day post-surgery corticosterone replacement therapy (5mg/kg body weight - .000165% corticosterone) was begun on all subjects. No change in activity was noted in the control group. A return to normal activity levels in the Adx group was obtained by the third day of therapy. The return to normal activity levels in the VMH group was immediate (within 24 hrs. of corticosterone replacement). By the end of 48 hrs. post-therapy the VMH Ss were running at levels significantly higher than their pre-surgery levels and significantly higher than either the Adx or control Ss on replacement.

These results suggest at least a two step relationship between VMH lesions and control of activity in the rat- - 1) VMH lesions interrupt the release of corticosterone from the adrenal cortex, and 2) VMH lesions disinhibit the S's response to corticosterone resulting in hyperactivity.

949 ACTIVITIES OF N-ACETYLSEROTONIN AND MELATONIN IN BIOLOGICAL TISSUES. G. M. Brown, S. F. Pang*, L. J. Grota , J. W. Chambers* and R. L. Rodman*. Clarke Institute of Psychiatry, Toronto, Ontario and University of Rochester, Rochester, N.Y.

It has been suggested that melatonin (M) and N-acetylserotonin (NAS), the two derivatives of serotonin, may function as hormones, neuromodulators or neurotransmitters. However, due to the lack of efficient methods for the quantitative determination of M and/or NAS levels, progress in M and NAS research has been slow. Immunization with M-bovine serum albumin (BSA) and NAS-BSA conjugates in rabbits produced anti-M and anti-NAS sera respectively. Anti-M serum reacts 100% with M and crossreacts 1.3% with NAS, 1.0% with 6-hydroxymelatonin and 0.1% with compounds of similar structure to M. Anti-NAS serum reacts 100% with M and NAS and crossreacts 10% with 6-hydroxymelatonin, 0.6% with 5-methoxytryptamine and 0.1% with compounds of similar structure to M. With these antisera, radioimmunoassays were developed to measure M and NAS in tissues. There are detectable levels of both M and NAS in pineals, Harderian glands, retinas and brains of rats and chickens, and of M in sera and urine of rats, chickens and human beings. In the rat pineal, there are diurnal rhythms of both M and NAS with high levels at night and low levels in the daytime. Pineal melatonin rhythm obtained in this study agrees well with those determined by bioassay. Pineal NAS rhythm found in our experiment correlates well with the diurnal rhythm of pineal N-acetylserotonin transferase. Radioimmunoassays described in this study would be important to future investigations on the function of M and NAS in pineals, Harderian glands, retinas and other tissues.

950 CHANGES IN STRUCTURE AND FUNCTION OF EPINEPHRINE STORAGE GRANULES FOLLOWING HYPOPHYSECTOMY. W.J. Burke*, V.W. Fischer, S. Horenstein, B.D. Bhagat*, J.W. Davis, Dept. Neurol., St. Louis U. Sch. Med., St. Louis, MO 63104.

The catecholamine content of rat adrenal glands is decreased after hypophysectomy (hypox). This may be partly due to a decrease in catecholamine synthesizing enzymes. However, treatment of hypox rats with ACTH over six days restored phenylethanolamine N-methyl transferase (PNMT) and tyrosine-hydroxylase (TH) activities but did not restore catecholamine content to control levels. To investigate directly the possibility that the storage capacity of catecholamine granules may be impaired after hypophysectomy, electron microscopic and biochemical studies were performed on cultured explants of adrenal medullary tissue.

The PNMT activity in medullary tissue from hypox rats was diminished 59% if the tissue was cultured 48 hr in a medium containing 0.5 mM epinephrine (epi). The PNMT of tissue from intact rats was reduced only 25% under the same conditions. The PNMT of tissue from hypox rats treated with dexamethasone had the same sensitivity to epi as hypox rats. Electron microscopic studies of medullary tissue from hypox rats showed large depleted granules. Storage granules in medullary tissue from hypox rats cultured in 0.5 mM epi remained degranulated. Structural changes in storage vessicles were not entirely reversed by injection of the animals with dexamethasone. The results suggest that hypox alters the storage capacity of granules in the adrenal medullary tissue and steroids do not reverse these changes.

951 EFFECT OF THYROTROPIN RELEASING HORMONE ON NOREPINEPHRINE UPTAKE IN RAT CEREBRAL CORTICAL HOMOGENATES. G. Howard Burrows*, Elaine S. Robinson* and Edith D. Hendley. Dept. Physiol. & Biophys., Univ. Vermont, Burlington, VT 05401.

Thyrotropin releasing hormone (TRH) has a number of actions on the central nervous system that resemble in many ways the effects of amphetamine. In light of the known inhibitory effect of amphetamine on norepinephrine (NE) uptake, it was considered important to study the actions of TRH on the NE uptake process. We tested TRH *in vitro* on the uptake of ^{3}H-l-NE in cerebral cortical homogenates from normal male rats. At concentrations from 10^{-12} to 10^{-4}M TRH failed to alter the 5 min uptake of ^{3}H-l-NE, 0.1 µM, except for minimal inhibition at 10^{-5}M or above. In contrast to *in vitro* TRH, when rats were pretreated with TRH (1 mg/kg ip) 45-60 min prior to killing and the kinetic constants for ^{3}H-l-NE uptake determined in cortical homogenates from these rats, it was found that the apparent K_m was significantly increased with no change in the V_{max} as a result of TRH administration. In addition, the inhibitory constants (K_i) were determined for desmethylimipramine (DMI) or d-amphetamine as inhibitors of ^{3}H-l-NE uptake in homogenates from TRH pretreated rats or control saline-injected rats. TRH pretreatment failed to alter the K_i for either DMI or d-amphetamine, and therefore presumably, did not alter the affinities of these inhibitors for the NE neuronal membrane uptake mechanism. We concluded that TRH did not alter NE uptake *in vitro* but that after pretreatment systemically TRH increased the apparent K_m without altering V_{max}, and without affecting the K_i for DMI or d-amphetamine. The possible involvement of the pituitary-thyroid axis with this action of TRH is currently under investigation. Supported by USPHS RO1-MH25811.

952 EFFECTS OF NEONATAL INTRACEREBRAL IMPLANTS OF ESTRADIOL OR TESTOSTERONE ON NEUROENDOCRINE FUNCTIONS IN FEMALE RATS. Larry W. Christensen* and Roger A. Gorski. Dept. Anatomy and Brain Res. Inst., UCLA Sch. Med., Los Angeles, Calif. 90024.

The effects of neonatal intracranial implants of testosterone (T), estradiol (E) or cholesterol (C) on adult masculine and feminine sexual behavior and on gonadotropin (GTH) cyclicity were assessed by implanting small pellets of T, E or C in the dorsal preoptic area (POA), ventral medial hypothalamus (VMH), anterior hypothalamus or mesencephalic reticular formation of female rats on day 2 or day 5 (day 1 is the day of birth). Dosages per pellet were 2 μg for T and C, 1 μg for E. Bilateral pellets of T or E, when placed in the VMH on day 2, but not into any of the other neural sites, significantly reduced the number of animals showing adult cyclic GTH patterns (as evidenced by corpora lutea). On the other hand, implants of T or E into the POA on day 2, but not into other neural sites, significantly increased the level of masculine sexual behavior exhibited in the adults under either estradiol benzoate (EB) or testosterone propionate therapy. The same implants of E or T placed in the POA on day 5, however, produced little if any increase in male sexual behavior. Day 2 implants of E, but not T or C, into the VMH significantly reduced the level of female sexual behavior exhibited by adults in response to EB plus progesterone therapy. These data indicate that E is as effective as T in masculinizing the hypothalamus. With respect to masculine sexual behavior, the apparent temporal "critical period" for perinatal hormone action in the dorsal POA appears to have ended by day 5. Moreover, these data suggest that different areas of the neonatal nervous system are involved in the sexual differentiation of sexual behavior (dorsal POA) and GTH cyclicity (VMH). Thus, the dissociation in the neonatal masculinization of neuroendocrine functions may have both a spatial and temporal basis. (Supported by USPHS Grant No. HD-01182.)

953 EFFECTS OF NARCOTICS ON SERUM LUTEINIZING HORMONE LEVELS IN THE MALE RAT. Theodore J. Cicero, E.R. Meyer* and R.D. Bell*. Dept. Psychiatry and Anatomy and Neurobiology, Washington U. Sch. Med., St. Louis, MO 63110.

Chronic morphine or methadone administration produces a marked decrease in serum testosterone and luteinizing hormone (LH) levels in the male rat and human. In these experiments, the effects of an injection of morphine (20 mg/kg) on serum LH levels were examined in the rat to determine whether the effects of narcotics on the hypothalamic-pituitary-gonadal axis represent specific effects of these drugs. One hour after the injection of morphine, serum levels of LH were significantly depressed below control levels and remained depressed, by more than 75%, for the next 4 hours. Six hours after the injection of morphine LH levels seemed to rebound slightly. The effects of the following narcotics were examined on LH levels: etorphine, etonitazene, Levorphanol, morphine, methadone, pentazocine and codeine. The results indicated that all narcotics depressed serum levels of LH and that their potency, relative to morphine, was comparable to that observed in several other preparations, such as inhibition of electrically-induced contractions of the guinea pig ileum and opiate-binding to brain homogenates. Also, the pharmacologically active levorotatory isomers of the narcotics were considerably more effective in depressing serum LH levels than were the inactive dextrorotatory isomers. Finally, naloxone competitively inhibited the effects of the narcotics on LH levels and tolerance developed to the LH-depleting effects of these drugs. On the basis of these findings, the reduction in LH levels by the narcotics appears to represent a specific narcotic drug affect. The mechanism underlying the narcotic's inhibition of LH levels is at present unknown but recent data suggest that the drugs probably act at some point in the hypothalamic-pituitary link of the hypothalamic-pituitary-gonadal axis.

954 NEUROENDOCRINE REGULATION OF ROTATIONAL BEHAVIOR IN NONLESIONED RATS BY THYROTROPIN RELEASING HORMONE (TRH), SOMATOSTATIN, LUTEINIZING HORMONE RELEASING HORMONE (LHRH) AND SUBSTANCE P. Marthe Cohn* and Major L. Cohn (Sponsor: R.G. Selker) Department of Anesthesia, Magee-Womens Hospital, Univ. of Pitt. Sch. of Med., Pittsburgh, Pennsylvania 15213.

Although many of the hypothalamic release inhibiting neurohormones are found throughout most parts of the brain, their physiologic functions in the CNS are not known. Ungerstedt (1971) linked rotational behavior to dopaminergic pathways in rats unilaterally lesioned in the nigrostriatal tracts. Our finding that centrally injected TRH produces similar rotational behavior in nonlesioned rats suggests that the neurohormone acts at the same, well-defined anatomical site in the brain. Supporting our contention is the fact that haloperidol and pimozide inhibit TRH-induced rotational behavior. We also reported that somatostatin produces "barrel rotation", which is inhibited by atropine. Thus, somatostatin seems to act through cholinergic mechanisms. In the present study, we determined whether LHRH, MIF, Substance P and glutathione produce rotational behavior in naive or Sprague-Dawley rats pretreated with apomorphine (6 mg/kg) or reserpine (6 mg/kg). Our findings show that in rats pretreated with apomorphine or reserpine, TRH, LHRH and Substance P-induced tight head to tail rotations are linked to dopamine transmission. However, in naive rats, somatostatin, LHRH and Substance P-induced "barrel rotations" are linked to acetylcholine transmission. In rats confined in a tight plastic rat chamber and unable to rotate, TRH, somatostatin, LHRH and Substance P produce nystagmus. The fact that nystagmus is produced by modulating the vestibular-ocular reflexes in the absence of locomotor activity of the head suggests that the neurohormones act at multiple receptor sites.

955 PLASMA CORTICOSTERONE LEVELS IN THE RAT AFTER AMYGDALOID ABLATION OR STIMULATION. J. D. Dunn and A.J. Carrillo. Depts. Anat., Loyola Univ. of Chicago, Stritch Sch. Med., Maywood, Ill. and Louisiana State Univ. Med. Sch., New Orleans, La.

Participation of the amygdaloid complex in pituitary-adrenal function was assessed by fluorometrically measuring plasma corticosterone (Cpd B) levels after a variety of ablative or stimulatory procedures. In male rats, transection of the stria terminalis, ablation of the basolateral amygdala or subtotal amygdalectomy did not alter AM or PM nonstress plasma Cpd B levels. Likewise stress-induced plasma Cpd B increments in experimentals were not different from controls after 3 min exposure to ether vapor or immobilization in a supine position. Similarly, in female rats neither bilateral ablation of corticomedial (CM) or basolateral (BL) amygdala produced alterations in nonstress or stress aspects of pituitary-adrenal function. As a corollary to the ablation studies, male rats bearing chronically implanted electrodes were subjected to sham or electrical stimulation. In non-anesthetized (freely moving) rats electrical stimulation of either CM or BL as well as the arcuate nucleus (ARC) resulted in plasma Cpd B levels significantly greater ($P<0.05$) than those in sham stimulated controls. In contrast, in Nembutal (50 mg/kg) anesthetized rats plasma Cpd B levels in CM and BL stimulated animals were not different from those of sham stimulated rats; only stimulation of ARC produced plasma Cpd B levels greater than ($P<0.05$) those in sham stimulated rats.

These data indicate that the amygdala is capable of influencing ACTH secretion. However, the finding that pituitary-adrenal function was not compromized by large amygdaloid lesions suggests that the amygdala is not essential for pituitary-adrenal function as we assessed this function.

956 IS LH RELEASE FOLLOWING ELECTROCHEMICAL STIMULATION OF THE MEDIAL PREOPTIC AREA DUE TO ENHANCED RELEASE OF LHRH? R.L. Eskay*, R.S. Mical*, and J.C. Porter. Depts. of Ob-Gyn & Physiol., Cecil H. & Ida Green Ctr. for Reproductive Biology Sciences, Southwestern Med. Sch., Dallas, TX 75235.

Electrochemical stimulation (100 μa × 60 sec) of the medial preoptic area (MPOA) of proestrous rats resulted in a significant release of LHRH into hypophysial portal plasma (HPP) and of LH into systemic plasma (SP). LHRH levels in HPP collected during the 0-30, 30-60, 60-90, 90-120, and 120-150 min periods after stimulation were 105 ± 24.2, 61 ± 10.8, 51 ± 8.2, 36 ± 5.3, and 32 ± 4.1 pg/ml, respectively (mean and SE; N = 24). Zero, 30, 60, 90, 120, 150, and 180 min after stimulation, LH levels in SP were 20 ± 4.0, 68 ± 10.9, 181 ± 52.2, 858 ± 104, 1240 ± 120, 936 ± 84.0, and 760 ± 70.0 ng/ml, respectively. In unstimulated animals, LHRH levels in HPP were less than 12 pg/ml in most rats. To determine whether changes in LHRH levels in HPP after MPOA stimulation were responsible for the increased release of LH, animals were infused i.v. with 100 μl of normal rabbit serum (NRS) or anti-LHRH serum prior to electrochemical stimulation. This volume of antiserum when given i.v. on the morning of proestrus was found to inhibit the LH surge during the evening of proestrus and to block ovulation in all animals. In addition, 100 μl of the antiserum given i.v. suppressed LH release in ovariectomized rats. When NRS (100 μl) was given i.v. to proestrous rats 2.5 min before stimulation of the MPOA, LH levels in SP rose from 95 ± 12.6 to 1890 ± 257 ng/ml within 2 hr. Anti-LHRH serum (100 μl) injected 2.5 min before electrochemical stimulation completely blocked LH release. On the basis of these findings, it is concluded that the increased release of LH that occurs following electrochemical stimulation of the MPOA is a consequence of an enhanced release of LHRH.

957 DEVELOPMENT OF A CIRCADIAN RHYTHM IN PINEAL N-ACETYLTRANSFERASE IN THE RAT M. Felong* and R.Y. Moore, Dept. Anatomy, Univ. of Chicago and Dept. Neurosciences, Univ. of California at San Diego.

The rat pineal gland exhibits a circadian rhythm in the activity of the enzyme, N-acetyltransferase (NAT), with peak values occurring during the dark period and trough values during the light period of a diurnal environmental lighting (LD) schedule. This circadian rhythm is a function of an endogenous oscillating, rhythm-generating mechanism which is entrained to the LD cycle by visual information reaching the CNS through the retinohypothalamic projection to the suprachiasmatic hypothalamic nucleus.

The present study was carried out to determine the relationship between the development of the retinohypothalamic projection, the development of the pineal NAT rhythm and its entrainment to the LD cycle. Autoradiographic studies demonstrate that the retinohypothalamic projection reaches the suprachiasmatic nuclei by the 4th postnatal day. A distinct circadian rhythm in pineal NAT is also evident at 4 days but not at three days postnatal in animals maintained in LD. Development of the NAT rhythm is suppressed in animals raised in continuous light after birth whereas animals blinded at 2 days or raised in continuous darkness exhibit evidence of development of a circadian NAT rhythm between the 4th and 6th postnatal days.

These observations indicate that the development of the endogenous oscillating mechanisms mediating this circadian function is paralleled by the development of the visual pathway necessary for its entrainment. (Supported by NIH Grant NS-12267)

958 MUSCLE PARAMETERS AS AFFECTED BY AN ANABOLIC STEROID. E.R.Gonzalez* and F.L.Strand. Dept.Biology. New York University. N.Y.10003. USA.

The influence of nandrolone phenpropionate N.F.(Durabolin) on muscle action potentials (APs) and isometric contractions was studied using the extensor digitorum longus (EDL) in situ in intact and castrate rats. The EDL was stimulated indirectly at a frequency of 10/sec;duration 0.05 msec strength 3x maximal, for 30 min. After 10 min rest, stimulation was resumed for 10 min. A fatigue curve was obtained for both periods. Post-tetanic potentiation was recorded 10 min prior to continuous stimulation, and at 30 and 50 min.Castrates were tested 1 and 2 months after operation.

Single injection experiments consisted of 1 injection of Durabolin (15 mg/kg) 3 days prior to testing. Chronic Durabolin treatment consisted of 1 injection every other day for 10 days. Testing followed 2 days after the last injection. All controls were oil injected.

A single injection of Durabolin had no effect on muscle tension(muscle contraction-volts-/calibration factor x muscle weight)of 1 mo.castrates but appeared to affect contraction time. Chronic treatment with Durabolin however, decreased muscle tension in normal rats and increased tension in 2 month castrates. In both cases, Durabolin had no effect on muscle APs or on post-tetanic potentiation.

These results indicate that chronic treatment with an anabolic steroid may affect the contractile properties of the indirectly stimulated muscle rather than neurotransmission, or the electrical characteristics of the muscle.

959 EFFECTS OF PROLACTIN AND HALOPERIDOL ON DOPAMINE TURNOVER IN THE TUBEROINFUNDIBULAR AND NIGROSTRIATAL PATHWAYS OF THE RAT BRAIN. G.A. Gudleksy, J. Simpkins*, J. Meites* and K.E. Moore. Depts. of Pharmacology and Physiology, Michigan State University, East Lansing, Michigan, 48824.

It has been previously suggested (Fuxe et al., 1969) that the tuberoinfundibular dopamine system is involved in the regulation of prolactin (PRL) secretion. The nigrostriatal pathway appears to be involved with extrapyramidal motor functions. The present studies were undertaken to assess possible differences in the regulatory mechanisms of these dopaminergic neurons. A radioenzymatic procedure was used to quantify the effects of PRL and haloperidol on dopamine (DA) turnover in the median eminence and striatum, regions containing terminals of the tuberoinfundibular and nigrostriatal neurons, respectively. Turnover was determined from the α-methyltyrosine (αMT)-induced depletion of the DA concentration. Female ovariectomized rats were injected with ovine PRL (5.0 mg/kg, s.c.) every 8 hr and sacrificed 2 hr after the last injection. One hour before sacrifice rats received saline or αMT (250 mg/kg, i.p.). PRL had no effect on DA concentrations in the median eminence or striatum. DA turnover in the median eminence but not in the striatum was increased after 26 and 74 hr of PRL administration. PRL had no effect in either region after 2 or 10 hr. Haloperidol (2.5 mg/kg, s.c.) increased DA turnover in the striatum but not in the median eminence 2 and 8 hr after injection. DA turnover was increased only in the median eminence 16 and 24 hr after haloperidol, an effect which was blocked by hypophysectomy. These data suggest that whereas activity of the nigrostriatal pathway may be modulated by a neuronal feedback system, activity of the tuberoinfundibular pathway may be modulated by a hormonal feedback system. (Supported by USPHS grant NS 09174.)

960 HORMONAL CONTROL OF HYPOTHALAMIC CELL NUCLEAR VOLUME IN FEMALE RATS. Shelton E. Hendricks and Robert J. Pickett*. Dept. Psychol, Univ. Nebraska at Omaha, Omaha, NE. 68101.

The nuclear volume of cell nuclei of the medial preoptic area (MPOA) and ventral-medial hypothalamus (VMH) were measured in adult female rats. These animals had been ovariectomized at birth or 60 days of age and injected with either corn oil vehicle, 5 µg testosterone propionate (TP) or 50 µg TP at three days of age. The nuclear volume of neurons within the MPOA decreased with increased dosages of TP. Neonatal ovariectomy, as compared to ovariectomy at 60 days of age, resulted in smaller nuclear volumes of neurons in subjects injected with either 5 µg TP or 50 µg TP but not in oil-injected animals. No differences were observed in nuclear volume of oligodendroglial cell nuclei within the MPOA with respect to TP treatment or age of ovariectomy. Within the VMH, the nuclear volume of neurons decreased as a function of TP treatment but there were no differences between the effects of the two dosages employed. Neonatal ovariectomy reduced nuclear volume in the VMH only within the oil groups. Results were interpreted as indicating that neonatal androgenization and the ovary present during prepuberal life cause permanent but opposite, selective structural changes within the hypothalamus of the female rat. Further, these changes were found to be remarkably similar to and consistent with the reported effects of these manipulations on reproductive physiology and behavior.

Supported by NIH Grant NS10027 (SEH).

961 AUTORADIOGRAPHIC LOCALIZATION OF ESTRADIOL IN CATECHOLAMINE NEURONS OF THE RAT BRAIN STEM. Aileen S. Heritage*, Lester D. Grant and Walter E. Stumpf Dept. of Anat. and Neurobiol. Program, Univ. of No. Carolina, Chapel Hill, N. C. 27514.

Combined fluorescence microscopy/dry-mount autoradiography methods were used to map estrogen uptake sites in relation to catecholamine (CA) neurons in medulla, pons, and midbrain of the rat. Female albino rats were injected with ^{3}H-estradiol-17β (0.5 or 1.0 µg/100 g b.w.) 48 h after ovariectomy and sacrificed 1 h later. Cryostat sections of brain cut at 4-6 µ were freeze-dried for 18-20 h, and either (1) single sections were exposed to formaldehyde gas for 1 h at 80° C and then processed for autoradiography, allowing for simultaneous visualization of silver grains in relation to fluorescing CA neurons, or (2) pairs of consecutive sections were collected, one for exposure to formaldehyde gas for demonstration of fluorescing CA neurons and the other for autoradiographic processing, thus allowing comparison of immediately adjacent sections. The same pattern of results was obtained with each procedure: (1) Silver grains indicative of estradiol uptake were concentrated in nuclei of cell bodies of scattered fluorescing CA neurons, e.g., in the caudal portion of group A1 norepinephrine (NE) cells in the ventrolateral reticular formation of medulla, in many A2 NE cells in the vicinity of nucleus tractus solitarius, and in some ventral cells of the A6 NE group in locus coeruleus. (2) No nuclear uptake of estradiol was observed in cell bodies of A8, A9 or A10 dopamine neurons in substantia nigra and adjacent areas of midbrain. (3) A few non-CA neurons with nuclear uptake of estradiol were observed in regions containing CA terminals, e.g., nucleus tractus spinalis nervi trigemini, nucleus tractus solitarius and central gray at caudal midbrain levels. (Supported by NIH grants NS09884 and NS09914.)

962 IMMUNOCYTOCHEMICAL LOCALIZATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH). DIFFERENCES WITH DIFFERENT ANTISERA. Gloria E. Hoffman, Jan A. Moynihan* and Karl M. Knigge*. Dept. Anat., Univ. Rochester Sch. Med., Rochester, NY. 14642.

There has been a great deal of speculation as to why LHRH cannot consistently be localized immunocytochemically within perikarya in the hypothalamus. The possibility that LHRH is contained in different antigenic forms in perikarya versus neuronal processes and terminals was examined. Alternate sections of Bouin's fixed mouse brains were reacted with either antisera generated against a tyrosine (or histidine) conjugate of LHRH (Niswender #42) or an arginine conjugate of the neuropeptide (Sorrentino antisera F2). The antisera from the tyrosine conjugate revealed staining in neuronal processes and terminals only. Staining using the antisera generated against the arginine conjugate revealed immunoreaction within perikarya in the arcuate region as well as in neuronal processes and terminals.

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963 HYPOTHALAMIC QO_2 AND DRY WEIGHT MEASUREMENTS IN THE NEONATAL RAT. Alan Kling* (SPON: M. HAMBURGH). Dept Biology, Vassar College, Poughkeepsie, N.Y. 12601 Current address: Emory University, Sch. Med., Atlanta, GA. 30322

Neonatal hypothalamic QO_2 and dry weight measurements obtained at 6, 12, 18 and 24 days of age have been shown to demonstrate a progressive rise. TRF concentrations, which remain at a constant level in the newborn and fetus at 20% of adult levels, rise progressively 3-7 days post partum and reach adult levels by Day 14: the hypothalamus, pituitary, and thyroid were also noted to mature almost simultaneously during this period. (Dussault and Labrie, 1975). The time of rise in TRF is of special interest in that deprivation of thyroid hormone during the first 10 days of life is known to exert an irreversible effect on nervous tissue development i.e. protein metabolism, myelinization, branching of the dendritic tree. A stress nonresponsive (SNR) period during which time the hypothalamo-hypophyseal-adrenal axis is refractory to stress is known to occur between days 3-12 (Schapiro et al, 1962). Ether stress fails to elicit increased synthesis and release of corticosterone in the 15 day old hypothyroid rat but is effective in the euthyroid animal: this nonresponsiveness is of hypothalamic orign (Meserve and Leathem, 1973). It thus appears that the postpartum period during which a progressive rise in hypothalamic QO_2 and dry weight measurements has been demonstrated corresponds to a period during which other organs may be dependent upon hypothalamic function (e.g. thyroid gland) and during which hypothalamic development is itself receptive to factors which affect its functional maturation and development.

964 EFFECTS OF OVARIECTOMY AND ESTROGEN ON REGIONAL CONCENTRATIONS OF HYPOTHALAMIC LRF AND PITUITARY LH SECRETION IN THE RAT. Ronald M.Kobayashi, K.H.Lu* and S.S.C. Yen*. Depts. of Neurosciences and Reproductive Med., University of California, San Diego, Sch. Med., La Jolla, CA 92093 and Neurology Service, VA Hospital, San Diego, CA 92161.

The influence of ovariectomy (OVX) and estrogen treatment on hypothalamic LRF and pituitary and serum LH was studied in female rats. Twelve specific hypothalamic nuclei, retrochiasmatic area (RCA) and organum vasculosum lamina terminalis (OVLT) were removed by microdissection for LRF determinations by RIA. In proestrus female rats, the highest LRF conc. was found in the median eminence (ME), with high LRF conc. also seen in the RCA and OVLT, confirming previous reports. OVX induced a 72% reduction of LRF in the ME and 89% reduction in the RCA but no change in the OVLT at 4 weeks. At 2 weeks after OVX, LRF in the ME was 39% reduced. The marked decrease in LRF in both the ME and RCA following OVX was accompanied by parallel increases in both pituitary and serum conc. of LH, suggesting increased LRF release. Daily treatment with estradiol benzoate (EB; 20 ug sc) for 2 weeks completely prevented these changes. This effect of EB on LRF and LH persisted for at least 2 weeks after EB withdrawal. No significant effect of OVX and EB were observed on LRF in the other 10 hypothalamic nuclei examined.

These results suggest that 1) hypothalamic LRF content of ME and RCA are subject to profound influence by ovarian steroids, 2) the parallel decrease in LRF content and increase in LH release imply accelerated LRF release in excess of synthesis and 3) LRF in the ME and OVLT respond differently to ovarian steroids, implicating disparate regulation of LRF in these 2 regions. (Supported by NIMH Grant-26072, VA Grant 500401, Rockefeller Grant RF-75029; RMK is a VA Clinical Investigator).

965 RESTORATION OF VAGINAL CYCLICITY AFTER LIGHT-INDUCED PERSISTENT ESTRUS IN THE RAT, AND THE EFFECT OF AMYGDALOID DEEFFERENTATION. Richard J. Krieg Jr. Departments of Anatomy, University of California, Los Angeles, CA. and Medical College of Virginia, Richmond, VA. 23298

The spontaneously ovulating female rat, when exposed to constant light of sufficient intensity, undergoes endocrine changes which result in a condition of persistent estrus, as evidenced by continuously cornified vaginal epithelium. The present studies were designed to examine the return of vaginal cyclicity after re-exposure of constant light-persistent estrous rats to a regular 14:10 light-dark schedule(LD). Adult female Sprague Dawley rats (Simonsen Labs, Gilroy, CA.) were exposed to constant light environment, and vaginal epithelium examined daily by vaginal lavage. Animals were considered to be in persistent estrus upon the occurrence of 7 consecutive days of cornified vaginal epithelium. At this time, one experimental group received bilateral "deefferenting" amygdaloid cuts as described previously (Endocrinology 97: 261, 1975). A sham operated group (N=25) and an intact group (N=10) served as controls. The recovery period was 24 days. While the initiation of persistent estrus required a prolonged period of time (35±3 days), the return to vaginal cyclicity in sham and intact groups was quite abrupt (1.95±.17 and 2.22±.14 days, respectively). Upon conversion to LD, animals would generally show 2 days of cornification, and subsequently assume regular 4 day cycles. Amygdala cut animals, however, were markedly deficient in their ability to regain vaginal cyclicity. Seven of 12 cut animals failed to resume cycling, and showed continuous vaginal cornification for up to two months after conversion to LD. These results demonstrate that the return of vaginal cyclicity after light induced persistent estrus is a relatively rapid phenomenon, and suggest that the amygdala may be a vital component of the functional mechanism. (Supported by A. D. Williams Fellowship at MCV and Eli Lilly Predoctoral Fellowship and NIH Grant NS01162 at UCLA).

966 EFFECTS OF INTRACISTERNAL CLONIDINE ON INSULIN SECRETION IN THE DOG. Marc L. Leavitt* and Ralph E. Miller. Dept. of Pharmacology, Sch. Med., University of Kentucky, Lexington, KY. 40506.

Clonidine decreases blood pressure (BP) and heart rate (HR) and alters peripheral plasma concentrations of growth hormone, glucocorticoids, and renin. These effects are believed to be secondary to an activation of C.N.S. alpha-adrenergic receptors.

We examined the effects of intracisternal clonidine (1µg/kg) on portal vein plasma immunoreactive insulin concentration, (IRI). Mongrel dogs of either sex were anesthesized with pentobarbital sodium (30 mg/kg) and artifically respired. The major arteries of the intestines and spleen were ligated; and the femoral arteries and veins of both legs were catheterized. The atlanto-occipital membrane was exposed and after a control period, clonidine, in a volume of 0.2ml normal saline (NaCl), was injected directly through it.

Clonidine (n=7) decreased BP and HR with nadirs at 34 min ($P<.005$) and 29 min ($P<.01$), respectively. Arterial glucose declined throughout the experiment. Portal vein IRI rose to 90±29% ($\bar{X}$±SE) above control levels at 15 min ($P<.025$). In 3 control experiments with injections of 0.2ml NaCl, glucose declined slightly and little change in BP, HR, and IRI occurred.

In four separate experiments using an innervated cross-perfused pancreas (Amer. J. Physiol. 230:(4),1976) intracisternal clonidine decreased HR and BP to lowest levels at 30 min (N.S.). Glucose declined and insulin rose throughout the experiments to its highest level at 79 min, 149±26% above control, ($P<.01$).

Conclusion: These data suggest that intracisternal clonidine increases insulin secretion, at least in part, by a direct neural effect on the pancreas. Supported by: NIH Grant AMI 17082-03; Kroc Foundation.

ACCELERATED DEVELOPMENT OF THE DIURNAL PITUITARY-ADRENAL RHYTHM BY PERINATAL THYROXIN TREATMENT. I. Lengvári*, B.J. Branch*, E. Zimmermann and A. Newman Taylor. Dept. Anat. & Brain Res. Inst., UCLA, Los Angeles, CA 90024.

Perinatal hormonal treatments are known to alter maturational processes of the central nervous system and affect development of several adaptational mechanisms of the organism. In this study litters of Sprague-Dawley rats were housed in a 14 hr light:10 hr dark schedule with lights on at 0400 hr. At 8, 9 and 10 days of age the rats were injected daily with 10 µg/g body wt thyroxin (T4) solution (1 mg/ml); the controls received the same volume of vehicle subcutaneously. Beginning at 14 days of age, blood samples were taken at 0800 (AM) and 1800 hr (PM) and their corticosterone (B) content was assayed fluorometrically. AM levels of B decreased and PM levels increased during development, such that significantly higher PM than AM plasma B levels were first found on day 15 in the T4-injected and on day 18 in the vehicle-treated animals. The AM-PM differences attained adult magnitudes on day 21 in the T4-injected rats and on day 28 in the controls; on day 28 treated animals did not differ from controls. Eye opening occurred 3 days earlier in the T4-treated animals (day 13 vs day 16 in the controls), as has been reported previously. The results suggest that T4 accelerates the maturation of neural structures which participate in the control of diurnal secretion of ACTH, presumably those which trigger the light-mediated synchronization of this rhythmicity. (Supported by Ford Foundation, NIH and IBRO/UNESCO.)

968 THE INHIBITORY EFFECT OF THE GABA DERIVATIVE β-(p-CHOLOROPHENYL)-GABA on ACTH RELEASE. G. Keith Matheson. Indiana Univ. Sch. Med., Evansville Center, Evansville, In. 47732.

The infusion of gamma-aminobutyric acid (GABA) into the rat third ventricle inhibits the release of ACTH, as measured by plasma glucocorticoid (GC) levels while the infusion of the GABA antagonist picrotoxin promotes the release of ACTH. This facilitatory effect of picrotoxin is blocked by the simultaneous infusion of GABA. This suggests that a GABA mediated inhibitory mechanism is part of the neural apparatus regulating the release of CRF in the hypothalamus. It has been demonstrated that the GABA derivative, β-(p-cholorophenyl)-GABA, (CP-GABA; Ciba-Geigy) mimics the effect of the parent compound on the presynaptic inhibitory mechanism of the spinal cord and that a therapeutic dose of CP-GABA produces almost total inhibition of the monosynaptic reflex for up to 8 hours. To test the effect of CP-GABA on the ACTH releasing mechanism, 8 cats were implanted with bipolar concentric recording/stimulating electrodes in the basolateral amygdala, hippocampus and ventromedial hypothalamus. Epidural electrodes were placed over the parietal and visual cortices for ECoG recording. An indwelling silastic jugular catheter was placed in each animal. Blood samples were collected and analyzed for GC content at 30 minute intervals for a duration of 4-6 hours. In animals with low plasma GC levels the injection of CP-GABA did not significantly alter basal secretion of ACTH. However, on those days when plasma cortisol levels were above 5µg/100ml plasma, CP-GABA administration produced a rapid decline in circulating GC's. CP-GABA was also able to inhibit the release of ACTH elicited by picrotoxin and the release of ACTH elicited by 30 minutes of intermitant electrical stimulation of the basolateral amygdala. (Supported, in part, by a grant from the H.J Conover research bequest to the Indiana Univ. Sch. Med.)

969 COMPARATIVE MORPHOLOGY OF SUPRAEPENDYMAL CELLS FOUND IN THE 3rd VENTRICLE OF THE RAT AND GUINEA PIG: A SCANNING ELECTRON MICROSCOPIC STUDY. J. A. Mitchell. Department of Anatomy, Wayne State University, Detroit.

Adult rats and guinea pigs were perfused with Karnowsky's aldehyde reagent and the walls and floor of the 3rd ventricle prepared in routine fashion for examination by scanning electron microscopy.

Two types of supraependymal(SE) cells were found in both male and female rats and guinea pigs(GP). Type I possessed morphological features suggestive of a neuronal character; Type II had characteristics suggestive of phagocytic activity.

In the rat, Type I cells occurred on the ventricular floor, had ovoid cell bodies ($\bar{x}$12x15µ diameter), were relatively smooth surfaced and usually had 2-3 processes. In the GP, Type I cells occurred on both the floor and walls of the ventricle, had irregular bodies ($\bar{x}$15x18µ), rough surfaces and numerous processes ($\bar{x}$=13). In both species, processes were uniform in diameter, were occasionally varicosed, had collateral branches, traveled considerable distances (>100µ), intertwined with processes from adjacent cells to form intricate networks, and penetrated the ependymal surface.

In the rat, Type II SE cells had irregular, ruffled cell bodies with 2-4 stout pseudopod-like processes; in the GP, cell bodies were compact, regular, smooth surfaced and had 2-9 thin processes. The processes in both species terminated in membranous expansions. Type II cells were abundant on the choroid plexus and occurred in the infundibular recess.

These observations indicate that the 3rd ventricle of the adult rat and guinea pig is populated by at least two types of supraependymal cells. Type I appears to be stationary, forms intricate networks of fibers over the ventricular surface, penetrates the ependyma, and may be neuronal. Type II appears to be mobile, possesses pseudopod-like processes and may be phagocytic. (Supported by NIH Grant RR-05387-13.)

970 ACUTE GLUTAMATE-INDUCED ELEVATIONS IN SERUM TESTOSTERONE AND LUTEINIZING HORMONE. J.W. Olney, T.J. Cicero, E.R. Meyer* and T. de Gubareff*. Dept. Psychiatry, Washington Univ. Sch. Med., St. Louis, Mo., 63110.

Glutamate (GLU), a neuroexcitatory amino acid readily enters the arcuate region of the hypothalamus (AH) and destroys AH neurons. Evidence that other excitatory structural analogues of GLU mimic the toxic effect of GLU on AH neurons, suggests that excessive excitation may be the mechanism of GLU neurotoxicity. The AH serves multiple neuroendocrine regulatory functions; thus, deletion of AH neurons by GLU treatment of rodents in infancy gives rise to sequelae such as obesity, skeletal stunting, reduced weights of the adenohypophysis, gonads and accessory sex organs and reduced pituitary content of growth, luteinizing and prolactin hormones. Such evidence from chronic experiments prompted the present effort to determine whether a single subtoxic dose of GLU might acutely influence serum levels of testosterone and luteinizing hormone (LH) in the male adult rat. Our rationale was that a dose of GLU below that required to kill AH neurons might nevertheless stimulate them to fire at increased rates and thereby disturb endocrine systems regulated by these neurons. Our finding of a biphasic rise in serum levels of both LH and testosterone (each hormone reaching peak elevations at approximately 15 min and 4-6 hrs following GLU administration) is consistent with the hypothesis that systemically administered GLU interacts with AH neurons to induce acute perturbations in neuroendocrine (LH-RH?) regulatory pathways. Since the pituitary-gonadal axis may not be the only endocrine axis affected by GLU, the present experiment should be extended to include a broad spectrum evaluation of endocrine parameters following acute GLU administration. [Supported by USPHS grants DA-00259, NS-09156, MH-38894 and MH-70180]

971 THE IN VIVO BINDING OF ESTRADIOL IN HYPOTHALAMIC CHROMATIN. K. Olsen* and R.E. Whalen (SPON: C.B.G. Campbell). Department of Psychobiology, University of California, Irvine, CA 92717.

In peripheral tissues, estrogen binds to target cell nuclei. Nuclear fractionation studies have shown that the nuclear binding is to the chromatin material. Within the brain, estrogen binds primarily to hypothalamic nuclei. However, there have been no previous reports of subnuclear binding in the neural tissues. Therefore experiments were performed to investigate estrogen's association with brain chromatin.

Ovariectomized rats were sacrificed at various time points following i.v. injection of ^{3}H-17β-estradiol. Hypothalamic and cortical chromatin were obtained by isolation of a crude nuclear pellet followed by the rupture of the nuclear membrane and further chromatin purification. The accumulation and retention of radioactivity in the chromatin from the hypothalamus and cortex were examined 15 min., 30 min., 1, 2, 4 and 24 hr. after the administration of ^{3}H-E2. Maximal binding was found in the hypothalamic chromatin 30 min. after the injection of the hormone and the levels progressively declined over time. No appreciable binding was found in cortical chromatin at any time point. In some studies, unlabeled estradiol was given 30 min. before the injection of the ^{3}H-E2. Pretreatment decreased estradiol retention in the isolated hypothalamic chromatin to the level of binding found in the cortical chromatin, while having no appreciable effect on the cortical chromatin binding. These data demonstrate that in the brain estrogen selectively binds to chromatin of the hypothalamus and the binding process is of limited capacity. The results suggest that estrogen may modulate behavior and gonadotropin secretion through its interaction with genomic processes. SUPPORTED BY PHS GRANT #HD-00893.

972 MELATONIN IN RAT PLASMA AND URINE: DAILY RHYTHMS AND EFFECT OF PINEALECTOMY. Yoshisuke Ozaki,* Harry J. Lynch,* Richard J. Wurtman and Michael A. Moskowitz. MIT, Cambridge, MA 02139

The concentrations of melatonin in plasmas and urines of intact and pinealectomized rats were measured by both radioimmunoassay (RIA) and bioassay techniques. In intact rats exposed to a daily 12-hr photoperiod, the plasma melatonin concentration (assessed by RIA) during the daily dark period (47 pg/ml) was more than 5-fold greater than during the light period (9 pg/ml). This marked cyclic variation was not observed in plasmas from pinealectomized rats (12 pg/ml in the dark period *vs* 9 pg/ml in the light period). A similar rhythmic pattern was observed in the rate of melatonin excretion by intact animals (1 ng/12 hr estimated by RIA and 0.8 ng/12 hr estimated by bioassay during the dark period *vs* 0.3 ng/12 hr estimated by RIA and 0.2 ng/12 hr estimated by bioassay during the light period). In pinealectomized rats, the rate of melatonin excretion was the same during both light and dark periods (0.3 ng/12 hr measured by RIA and 0.1 ng/12 hr measured by bioassay). These findings suggest that the pineal is the major source of circulating melatonin, and that it secretes melatonin with a characteristic daily rhythm. However, the pineal may not be the exclusive source of melatonin in the rat.

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973 DIFFERENCE BETWEEN SERUM TESTOSTERONE LEVELS OF MALE AND FEMALE NEONATAL RATS DURING THE PERIOD OF NEURAL SEXUAL DIFFERENTIATION. S. F. Pang*, A. R. Caggiula*, V. L. Gay* and R. L. Goodman* (SPON: J. Seggie). Clarke Institute of Psychiatry, Toronto, Ontario, Dept. Psychology and Dept. Physiology, Univ. of Pittsburgh, Pittsburgh, Pennsylvania.

The role of sex hormones in neural sexual differentiation was studied by measuring the endogenous levels of testosterone, estrogen, LH and FSH in neonatal rats during the critical period of neural sexual differentiation. Male and female pups were sacrificed 1-5 days post-partum and the levels of sex hormones in the serum was determined by radioimmunoassay (RIA). At all five sampling times, the serum concentration of testosterone in male rats were about three times higher than that in female rats, but serum estrogen levels of the two sexes were about the same. Serum LH and FSH concentrations were lower in males than in females. In the second study, rats were decapitated 1-10 days after birth and serum testosterone levels was determined by a different testosterone RIA. Again, at all four sampling times, male serum testosterone levels are consistently higher than that of the female. Assuming that our testosterone assays provided a true level of testosterone in the serum samples, our observations agree well with the testosterone androgenization hypothesis of neural sexual differentiation. However, the finding that females have measurable testosterone suggests that either (1) a minimum level of the androgen must be present for androgenization to continue during the early postnatal period and that males exceed this threshold but female do not, or (2) androgenization is a dose dependent phenomena and female rats are normally, partially androgenized.

974 EFFECTS OF GROUP CAGING ON REPRODUCTIVE FUNCTION IN INTACT AND AMYGDALOTOMIZED MICE. James N. Pasley and Ervin W. Powell, Dept. Physiology and Dept. Anatomy, Univ. of Ark. for Med. Sciences, 4301 W. Markham, Little Rock, AR 72201.

Intact, amygdalotomized and sham-operated wild-derived brown house mice (Mus musculus) of both sexes were caged singly or in groups of 8-10 for three weeks. Ovarian and uterine weights of intact and sham-operated grouped female mice were significantly depressed compared to singly caged controls. Reproductive organ weights of grouped amygdalotomized female mice were not different from singly caged controls. Seminal vesicle weights of intact and sham-operated grouped males were significantly depressed compared to singly caged controls and to grouped amygdalotomized male mice. Plasma LH values from the various experimental groups coincided with the morphological data. The results of this study lend further support to the hypothesis that increased population density impairs reproductive function in mice. Moreover, perhaps the effects of decreased pituitary-gonadal function associated with grouping are mediated by amygdalar nuclei.

975 Distribution of Luteinizing Hormone-Releasing Hormone (LH-RH) in the Brain of the Adult Golden Hamster. G.E. Pickard* and A.J. Silverman (SPON: P. Claude). Wisc. Reg. Primate Res. Ctr., Univ. Wisc., Madison, Wisc. 53706 and Dept. Anat., College P&S, Columbia Univ., N.Y.,N.Y.10032

The distribution of LH-RH was studied in the brains of adult male and female hamsters. Using the unlabeled antibody enzyme technique (peroxidase-antiperoxidase complex supplied by L.A. Sternberger) and specific antiserum to LH-RH (provided by S. Sorrentino,Jr.), immunoreactive perikarya were localized primarily in the arcuate nucleus and in the immediately adjacent regions. Immunodeposits were not present in magnocellular neurons. Methodological and immunological controls verified the specificity of the reaction products.

LH-RH positive fibers were more widely distributed. Immunoreactive axons were scattered throughout the medial basal hypothalamus and projected to the median eminence where they were observed throughout the zona externa. A more diffuse fiber system traveled between the medial septal region and the arcuate nucleus passing on the pre-commissural side of the anterior commissure. A group of post-commissural axons coursed dorsally through the periventricular region of the thalamus and extended into the medial habenular nucleus. LH-RH axons were observed in the fasciculus retroflexus between the habenula and the interpeduncular nucleus. Reaction products were also found in the organum vasculosum of the lamina terminalis and the subfornical organ. A few fibers were seen near midline running along the base of the brain into the anterior olfactory nuclear area. In all regions, immunoreactive fibers were few in number.

LH-RH has been demonstrated in cell bodies of the arcuate nucleus and in a diffuse network of fibers distributed throughout the brain. It is not yet possible to specify all the sites of LH-RH axon terminations nor the perikaryal origin of the immunoreactive fibers described in this study.

976 REPRODUCTIVE PROCESSES AFTER EARLY MODIFICATION OF CENTRAL MONOAMINES IN FEMALE RATS. Nancy S. Pilotte* (SPON: Karen K. Glendenning). Dept. of Psychology, Florida State University, Tallahassee, FL, 32306.

Monoamine precursors or depletors were administered to neonatal female albino rats to assess the roles of serotonin and the catecholamines in the development and maintenance of reproductive function. Treatments were given on postnatal days 2 or 2 + 5. In rats given 5-hydroxytryptophan (5HTP, 3 mg/kg sc) on days 2 or 2 + 5 or 3,4-dihroxy-L-phenylalanine (DOPA, 10 mg/kg sc) on days 2 + 5, vaginal opening occurred at 35.8, 36.0 and 34.2 days, respectively, compared to 38.5 days for saline-treated controls. Effects of early treatment with these drugs on adult reproductive behavior were assessed by calculating daily lordosis quotients (LQ) during 3 consecutive vaginal estrous cycles for each group. Mean LQs obtained on the evenings of proestrus and estrus for control rats were 1.00 and 0.20, respectively. Reserpine-treated rats had proestrous and estrous LQs of 0.38 and 0.04. Proestrous and estrous LQs of rats given 5HTP on days 2 or 2 + 5 were 1.00 and 0.60 for the former and 0.03 and 0.14 for the latter. Proestrous and estrous LQs of rats treated with DOPA on days 2 or 2 + 5 were 0.95 and 0.30 for the former and 0.12 and 0.92 for the latter. Rats given reserpine or 5HTP (2 + 5) showed mounting behavior with pelvic thrusting on the evening of estrus. No differences were found between groups in the lengths of vaginal cycles recorded between 120 and 200 days of age. Both groups treated with 5HTP, however, often had cycles with a shortened proestrous phase (quiet estrus). The results indicate that early administration of agents which alter central monoamine concentrations influences tne development of normal reproductive function in rats.

977 EFFECT OF SALINE INFUSION INTO THE THIRD CEREBRAL VENTRICLE ON EXCRETION OF SODIUM IN THE CAT. L.R. Pryor*, T.I. Koike* and W.M. St. John. (SPON: E.A. Lucas). Dept. Physiol., Univ. Ark. for Med. Sci., Little Rock, AR 72201 and Dept. Pharmacol., Columbia Univ. Coll. Phys. and Surg., New York NY 10032.

Experiments were performed on 21 salt-loaded, chloralose anesthetized cats to examine changes in renal Na excretion resulting from the infusion into the third cerebral ventricle of 0.15 M and 0.85 M NaCl. Infusion of 100 ul of 0.85 M NaCl into the ventricle elicited two statistically significant natriuretic responses (q test, $P<0.05$). Significant increases in urinary Na excretion occurred at 20 min and at 190 through 270 min after the onset of the intraventricular infusion. The maximum value of the first natriuretic response was 417% of control. The second was 545% of control. The latter value occurred at 240 min post-infusion. Urinary K excretion was significantly elevated at 240 min (162% of control). Urinary Na:K ratios were elevated significantly at 20, 30, and 220 through 270 min. Maximal values for the ratios were 335 and 395% of control and occurred at 20 and 240 min, respectively. No significant changes in urine flow were observed. Sham infusion in 6 cats did not elicit any significant changes in Na excretion. The only changes exhibited in 6 cats subjected to intraventricular infusion of 100 ul of 0.15 M NaCl was an intermittent depression of Na excretion occurring from 160 to 300 min. Across-groups comparisons by U test ($P<0.05$) were essentially in accord with the within-groups findings. This analysis did not, however, support the findings of variations in the isotonic group. It is tentatively proposed that infusion of hypertonic NaCl into the third ventricle activates at least two natriuretic mechanisms. The mechanism responsible for the first natriuretic response have not been clearly resolved. The delayed natriuresis is consistent with a suppression of aldosterone secretion.

978 NEUROENDOCRINE CONSEQUENCES OF DAILY ACUTE MELATONIN INJECTIONS VERSUS CHRONIC SUBCUTANEOUS MELATONIN DEPOSITS. R.J. Reiter, Dept. Anat., The Univ. of Texas Health Sci. Ctr. at San Antonio, San Antonio, Tx. 78284.

Daily melatonin injections given early in the light period to hamsters kept in long daily photoperiods (more than 12 hours light per day) have little influence on reproduction. However, Tamarkin et al. (Proc. 5th Ann. Mtg. Soc. Neuroscience, Abstr. 712 p. 458, 1975) have shown that daily melatonin injections given 10 hours after lights on are strongly inhibitory to reproduction. We confirmed these findings, namely, that melatonin given late in the light period has a marked inhibitory effect on testicular and accessory sex organ weights and on pituitary LH and prolactin levels. The daily dosage of melatonin was 25µg. Melatonin per se does not seem to be responsible for gonadal involution, since if hamsters are pinealectomized, melatonin fails to suppress sexual physiology. Likewise, if the pineal is surgically denervated, melatonin, even when administered near the end of the light period, does not suppress reproduction.

The chronic subcutaneous implantation of melatonin-beeswax pellets into hamsters causes effects essentially opposite to those of acute injections of the indole given late in the light period. Beeswax pellets containing melatonin (50µg to 1mg) implanted every two weeks into light deprived hamsters prevent the pineal gland from inducing gonadal regression. Finally, the presence of a subcutaneous melatonin-beeswax pellets prevents the gonad inhibiting effect of acute melatonin injections given late in the light period. (Supported by NSF Grant No. BMS 74-06276)

979 EFFECT OF PINEAL STALK LESIONING ON PLASMA CORTISOL AND ANDROGEN LEVELS DURING AND AFTER EXPOSURE TO COLD. Robert W. Rivest*, K.D. Roberts* and Franco Leporé* (SPON: M. Ptito). Dépt. Psycho., Univ. de Montréal & Dépt. d'Endocr., Hôp. Maisonneuve, Montréal, Canada.

In order to test the hypothesis that the pineal gland regulates other endocrine activity during stress, plasma cortisol and androgen levels were determined during and following cold exposure (7°C). Two groups of rabbits, one sham-operated (n=8) and the other with a severed pineal stalk (n=8) were implanted with a carotid cannula: blood samples were withdrawn prior to exposure to cold, at different times during cold exposure and after returning the animals to ambient temperature.

The results indicate that prior to exposure of the animals to the stressful situation, no difference in cortisol levels and no difference in androgen levels existed between the two groups. During cold exposure, plasma cortisol levels in lesioned animals were about twice those of control animals; androgen levels exhibited greater individual variations but controls tended to exhibit a maximum output at the end of the cold period (t=120 mn) whereas the lesioned animals showed a nadir at this time.

Following return to ambient temperature, cortisol levels of controls fell rapidly to baseline, while those levels decreased more slowly in lesioned animals. Androgen levels in both groups kept fluctuating with a slow stabilisation by the end of the experiment (t=240 mn).

These results indicate that the pineal is involved in the control of the response of the organism to stressful situations; however, when the general endocrine response returns to a tonic activity, the role of the pineal gland would appear to be of a secondary nature in the regulation of this response.

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980 CIRCHORAL PULSES OF PROSTAGLANDIN $F_{2\alpha}$ AND LUTEINIZING HORMONE IN THE CEREBRAL CIRCULATION OF OVARIECTOMIZED SHEEP. J. S. Roberts* and J. A. McCracken* (SPON: E. Klaiber). Worcester Foundation for Experimental Biology, Shrewsbury, MA. 01545.

Simultaneous sampling of carotid arterial and jugular venous blood of conscious, ovariectomized sheep has revealed that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) appears on the venous side of the cerebral circulation in circhoral pulses. Circhoral variations in circulating luteinizing hormone (LH), which also occur in these ovariectomized animals, can be related to the pulses of $PGF_{2\alpha}$ by a model which assumes that (1) each pulse of $PGF_{2\alpha}$ begins with a brief period of $PGF_{2\alpha}$ release into a cerebral compartment accessible to the bloodstream, (2) at the same time, LH is released as a burst from the anterior pituitary and (3) $PGF_{2\alpha}$ diffuses from the cerebral compartment into the bloodstream according to a first-order exponential decay process. Data from 7 experiments were fitted to this model. In each, regression analysis confirmed that the exponential disappearance of $PGF_{2\alpha}$ from the cerebral circulation is linear. Moreover, the average half-life of 22.8±1.21 min (mean±sem, N=87) agreed well with experiments in which $PGF_{2\alpha}$ was infused via the carotid artery for 60 min. After the infusion, $PGF_{2\alpha}$ disappeared from the cerebral circulation with a half-life of 19.4±0.8 min (mean±sem, N=3). Because the secretion of LH appears to be under aminergic control by the brain, and because PG's seem to be produced in connection with adrenergic neural activity, we interpret our findings to mean that the pulses of $PGF_{2\alpha}$ we measure reflect periods of intense activity in the "adrenergic brain".

981 EFFECTS OF 6-HYDROXYDOPAMINE ON THE INITIATION AND MAINTENANCE OF MATERNAL BEHAVIOR IN THE RAT. Phyllis A. Rosenberg and Howard Moltz.* Dept. Biopsyc., U. of Chicago, Chicago, Ill. 60637.

The catecholaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), was used to test the hypothesis that increased transmission across selected noradrenergic synapses is involved in the initiation but not the maintenance of maternal behavior in the rat. Specifically, primiparous female rats received intraventricular infusions of 6-OHDA (200 µg/20 µl) either 2 days prior to parturition, before the initiation of maternal behavior, or on postpartum day 4, after maternal behavior had been established. Control animals were infused with the vehicle alone. Fluorometric analysis indicated that 6-OHDA depleted hypothalamic norepinephrine (NE) in both prepartum and lactating females, although the degree of depletion was variable. Among prepartum animals, NE depletion of more than 30 percent of control levels caused a significant disruption in maternal behavior whereas among lactating animals, similar degrees of NE depletion had no significant effect on maternal displays. Thus, NE appears to be involved in the initiation of maternal behavior but not in the maintenance of the behavior once it is established.

982 EFFECTS OF INTRAVENTRICULAR P-CHLOROAMPHETAMINE ON LACTATION. David L. Rowland, Marianne K. Steele, and Howard Moltz*. Dept. Behavioral Sciences, University of Chicago, Chicago, Ill. 60637.

Previous studies have suggested serotonergic mediation of the suckling-induced release of prolactin (PRL) in the rat. In the present study, p-chloroamphetamine (PCA), a selective serotonergic neurotoxin, was infused into either lateral or third ventricle of rats beginning on Day 4 lactation, and the effects on both serum prolactin and lactation were analyzed. All PCA-treated animals, regardless of the site of infusion, showed significantly lower levels of serum PRL when compared with vehicle-injected controls. Furthermore, the effect on lactation was indicated by the exceedingly high mortality rate in litters of drug-infused mothers. Since there were no differences in the amount of suckling received by either experimental or control mothers, this factor could not account for the different levels of PRL. Nor could the high pup mortality in litters of PCA-infused mothers be explained by differences in maternal responsiveness.

Although both lateral- and third-ventricle infusion of PCA significantly depressed serum prolactin, and consequently lactation, it was the latter infusion site that resulted in the most profound effect. Yet, even at this site, PCA inhibition could be overcome by simultaneous administration of prolactin (subcutaneous). In such prolactin-injected animals, pup survival approached that of control litters. Our findings indicate the importance of brain serotonin in the mediation of PRL release and, hence, in the control of lactation itself.

983 IN VITRO PRODUCTION OF N-ACETYLINDOLEAMINES BY THE PINEAL GLAND. J. Ruse*, G. Brown and R. Rodman* (SPON: L.J. Grota). Faculty of Medicine, University of Toronto, Toronto, Canada.

In vitro production of N-acetylindoleamines (NAI) by pineal cell cultures has been examined. Pineals from adult male rats were treated with trypsin and DNase followed by collagenase and hyaluronidase. Dispersed cells were cultured on glass coverslips in Ham's F10 nutrient medium. Confluent monolayers were established in 4-5 days. Cells were rounded, with ovoid nuclei, prominent nucleoli and were lightly granulated. In older cultures these cells were dispersed among very long spindle-shaped cells. Yield per gland ranged from $4x10^{-4}$ to $9x10^{-4}$ cells. In a typical study cells were subcultured after 6 days and used 4 days later. Radioimmunoassay for NAI was done using an antiserum that binds melatonin and N-acetylserotonin equally. Cells exposed for 24 hr. to $3x10^{-4}$M norepinephrine (NE) showed a reproducible response pattern with no change in NAI until 6 hr. and a maximal response (usually about 10ng/ml) at 24 hr. After removal of NE release of NAI dropped sharply. Dose response relationships examined over a range of $1.5x10^{-8}$M to $3x10^{-4}$M NE demonstrated an increase in NAI production to a maximum at doses ranging from $3.0x10^{-5}$M to $1.0x10^{-4}$M, followed by a sharp decline at higher doses. When cells were maintained for 3 days with various concentrations of NE and then exposed to $3x10^{-4}$NE, response to the latter concentration was modified by the preceding treatment. It is concluded that this *in vitro* system can be useful in the study of the pharmacologic and hormonal regulation of NAI production by the adult pineal gland.

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985 DOPAMINE LEVELS IN RAT DIENCEPHALIC CELL GROUPS: ALTERATION BY ENDOCRINE MANIPULATION. W. J. Shoemaker and M. Schlumpf. NIMH, St. Eliz. Hosp., Washington, D.C. 20032.

We have previously demonstrated that the 4 diencephalic dopamine-containing cell groups (anterior hypothalamic, A14; dorsal hypothalamic, A13; arcuate nucleus-median eminence, A12; caudal hypothalamic, A11) are resistant to the catecholamine-depleting effects of 6-OH-dopamine. By measuring the dopamine content after microdissection and by glyoxylic acid-induced fluorescence microscopy, the cell bodies and processes of these cell groups remained intact compared to other dopamine-containing brain nuclei (A9, A10 & caudate nuc.). Several lines of evidence indicate possible endocrine functions for these cell groups. Toward that end, we have utilized a number of endocrine manipulations to test whether any of these regions would respond by altering their dopamine levels. The manipulations are: thyroidectomy, castration, adrenalectomy, lactation, lactation during a low-protein diet, ovariectomy, and water deprivation. In the A14 region, lactation resulted in a 30% dopamine (DA) decrease; when lactation was accompanied by feeding a 8% protein diet, the deficit was 50%. Water deprivation, on the other hand, resulted in an increase in DA of 130%. None of our treatments significantly changed the DA levels in either A11 or A13. In the arcuate nucleus-median eminence region (A12), all of the treatments lowered DA (except water deprivation), but only hypophysectomy (50%) and lactation on 8% diet (70%) were decreased significantly. The lack of a conventional uptake mechanism, their position relative to the 3rd ventricle, and their responses to differing endocrine states suggest a neurosecretory function for some of these cell groups. The secretory product could be the catecholamine, alone or in combination with another substance.

986 VASOPRESSIN RELEASE IN RESPONSE TO ACETYLCHOLINE BY ORGAN CULTURED RAT HYPOTHALAMIC-NEUROHYPOPHYSEAL SYSTEM. Celia D. Sladek and Karl M. Knigge (SPON: D. H. Van Dyke). Dept. of Anatomy, Univ. of Rochester School of Medicine, Rochester, NY. 14642.

The organ cultured hypothalamic-neurohypophyseal system (HNS) has been used as an in vitro system for studying control of the magnocellular neurosecretory system. These organotypic explants include the supraoptic nucleus, median eminence, and neural lobe. The presence of viable supraoptic neurons in HNS explants maintained up to 9 days in culture has been confirmed by evaluating the histological and neurosecretory characteristics of the preparations. The HNS releases vasopressin (AVP) at a constant rate in vitro and retains osmotically sensitive components as demonstrated by the stimulation of AVP secretion following addition of hypertonic NaCl to the culture media. Addition of 10^{-5}M acetylcholine to the culture media increases the rate of AVP secretion to 3 times control levels. This response of the HNS explant remains stable from day 3 to day 5 of culture. Furthermore, it is dose-dependent being 200% of control at 10^{-7}M and plateauing at 10^{-4}M acetylcholine. Hexamethonium blocks acetylcholine stimulation of AVP secretion, thus demonstrating the nicotinic nature of the cholinergic receptor. These studies characterize the effect of acetylcholine on AVP secretion which may in turn mediate the osmotic control of AVP secretion.

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987 GLYOXYLIC ACID-INDUCED HISTOFLUORESCENCE IN NORMAL AND SURGICALLY ISOLATED RAT MEDIAL BASAL HYPOTHALAMUS (MBH). C. Turpen, Y. Cheung*, L. Shonk*, and J.R. Sladek, Jr. Dept. Anat., Univ. Rochester Sch. Med., Rochester, NY 14642.

Glyoxylic acid-induced catecholamine fluorescence was examined in the MBH (with emphasis on the median eminence) of adult male rats. Intense, fine-sized varicosities were observed in the external layer, and fine linear profiles of varicosities were seen coursing through the ependymal and fibrous zones, suggestive of juxtaposition to tanycytes. Possible sources of origin for the latter pattern include 1) components of the ventral noradrenergic system, and 2) intrahypothalamic areas such as dorsal periventricular (A-14) and arcuate (A-12) neurons. In order to determine the origin of these terminals, male rats were subjected to complete isolation of the MBH using an Halasz-Pupp knife. As rapidly as 24 hrs after this "deafferentation," degenerative axon profiles were observed dorsal, as well as anterior and lateral, to the knife track. By 3 days postoperatively, fluorescence in both fibrous and external layers was reduced, compared to patterns observed in intact rats. Seven days after surgery, fine-sized (presumably regenerating) fibers were seen passing through the deafferentation wound. The results indicate that although fibers innervating the isolated area originate external to the MBH, the site of origin for the fine linear profiles of the fibrous zone are perikarya of the arcuate and rostral periventricular regions. These fibers appear to be capable of morphologically demonstrable rapid regeneration. The latter observation bears possible functional implications in assessing endocrine regulation following MBH isolation of the type employed in this study.

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988 THE TIME COURSE AND REVERSIBILITY OF ULTRASTRUCTURAL CHANGES IN THE NUCLEUS CIRCULARIS IN DEHYDRATING RATS. Charles D. Tweedle and Glenn I. Hatton, Depts. of Biomech. and Zool. and Dept. of Psych., Mich. State U., E. Lansing, MI 48824.

In a previous quantitative ultrastructural study, Tweedle and Hatton (BRB 1: 103, 1976) found that there developed an increase in the amount of direct soma-somatic contact between neurosecretory cells of the circularis and supraoptic nuclei of the rat following water deprivation. This apparently occurred through withdrawal of thin astrocytic processes from between the somata. To investigate the time course and reversibility of this phenomenon, rats were just dehydrated for 4 or 12 hrs. or alternatively dehydrated for 24 hrs. followed by rehydration for 12 or 24 hrs. (N=5 per group). The animals were then perfused and the circularis nuclei examined and quantified ultrastructurally. A significant increase in amount of direct soma-somatic contact was seen by 12 hrs. of dehydration. Following an initial 24 hrs. of dehydration, the amount of direct membrane apposition returned to normal levels by 24 hrs. but not 12 hrs. of subsequent rehydration. The somata were seen to be once again more extensively separated by glial processes. The greatest changes seen in the vesicle population of the cells were among the larger neurosecretory granules (1600-2400 Å) and lysosomes, both of which accumulated after 24 hrs. of rehydration. [NIH grant # NS-09140].

989 HYPOTHALAMIC DEAFFERENTATION: EFFECTS ON PULSATILE ANTERIOR PITUITARY (AP) HORMONE SECRETION. John O. Willoughby*, Leon C. Terry*, Paul Brazeau * and Joseph B. Martin. (SPON: Donald G. Lawrence). Dept. Neurology, McGill University, Montreal, Que.

Five hour AP hormone secretory patterns, using a 15 minute sampling frequency were obtained in unrestrained male rats subjected to Halasz-type hypothalamic deafferentations and chronically implanted with right atrial cannulae. Changes in the characteristic secretory profiles of radioimmunoassayable growth hormone (GH), prolactin (PRL) and thyroid stimulating hormone (TSH) were observed. In rats with complete deafferentation (CC) increased GH secretion occurred as indicated by an increased frequency of GH bursts and elevated GH trough values, even though they showed growth failure, as well as aggressiveness, hyperphagia and diabetes insipidus. Selective anterior cuts (AC) through the suprachiasmatic nucleus (SCN), and posterior cuts (PC) through the mammillary bodies did not affect the periodicity of GH bursts but GH secretory episodes occurred with random onset times with AC, indicating loss of light-dark cycle (LD) entrainment. With CC, PRL values remained continually low, while TSH bursts were largely abolished. Hypothyroidism was present in all animals with CC, proven by thyroxine determination (< 5ng/ml). Episodic TSH and PRL secretion were unaffected by AC or PC.

These results suggest that the isolated medial basal hypothalamus (MBH) contains neural substrates for episodic GH secretion and for tonic suppression of PRL secretion. Lateral connections to the MBH are necessary for normal TSH and PRL secretion. Visual pathways subserving GH LD entrainment pass through the region of the SCN. Hypothyroidism following CC could account for growth failure observed in these rats, while GH hypersecretion is hypothesized to result from surgical damage to the inhibitory component of the MBH mechanism subserving GH pulsatile release.

Neuroethology

990 A NEUROETHOLOGICAL STUDY OF DISPLAY BEHAVIOR IN LIZARDS.
Neil Greenberg, J. Leland Ferguson* and Paul D. MacLean. Laboratory of Brain Evolution and Behavior, NIMH, Bethesda, Md. 20014.

The present study was undertaken to test the effects of lesions of the paleostriatum on the display behavior of the lizard Anolis carolinensis. An ethogram of the social behavior of this reptile has revealed 42 postures or actions, several of which are incorporated into four types of displays. The two display types focused upon in this study were (1) a display associated with arousal ("signature" or "assertive" display) characterized by cadenced "pushups" and an extension of the throat fan; and (2) a territorial "challenge" display combining features of the arousal display with profile changes that increase the apparent size of the lizard.

The pre- and postoperative display tests are systematically conducted over a period of weeks on single adult males living in a chamber that allows controlled exposure to another adult male.

A special stereotaxic device and microtechniques are employed for placing electrocoagulative lesions in the forebrain. Since there is a nearly complete decussation of the optic tract, a lesion is placed in one hemisphere and the lizard's display tested with a patch alternately placed on either eye. One major advantage of this method is that it results in no apparent disturbance of thermoregulation.

To date, observations have been made on eight animals. Five lizards with unilateral lesions of the paleostriatum showed statistically significant deficits in their "challenge" display when looking through the eye contralateral to the lesion. Three of these animals retained the arousal display. Two animals with lesions of the dorsal ventricular ridge and a control in which an electrode was introduced without electrocoagulation showed no significant deficits in their "challenge" or "signature" displays.

This work complements investigations which showed that lesions of the medial segment of the globus pallidus or its main efferent pathways affected the species-typical display behavior of squirrel monkeys (MacLean, Fed. Proc. 32:384, 1973; Trans. Amer. Neurol. Assoc. 100:110, 1975).

991 Smooth and Saccadic Head Movements in Mantids. James Y. Lea and Conrad G. Mueller* (SPON: Dolores M. Schroeder). School of Medicine and Center for Neural Sciences, Indiana University, Bloomington, Ind.

Praying mantids possess two distinctive types of head movements which are utilized in orienting to salient objects in the environment. A novel target of appropriate dimensions entering the field will often elicit an initial orienting saccadic head movement. We have previously shown that these saccades are rapid (occurring in about 120 msec) and are not dependent upon visual feedback from the target. If the target continues to move across the field, the mantis will visually track it by making periodic saccadic movements. Smooth pursuit head movements are not seen in these circumstances.

Head movements may also be elicited by slowly moving a large portion of the environment with respect to the mantis. This procedure first produces a smooth head movement which serves approximately to "stabilize" the image of the world on the retina. When the head deviates more than 15° to 20° from midline, a compensatory saccade occurs in the opposite direction, followed once again by smooth movements. This sequence of behaviors is quite analogous to optokinetic nystagmus (OKN) seen in vertebrates.

In experiments reported here a mantis was placed in the center of a turntable around which was located a fixed environment consisting of black and white stripes of variable spatial frequency. An initial saccade was elicited by presenting a small moving object to one side of the mantis. After an orienting saccade was obtained, the target was rapidly removed so that the head orientation remained unchanged. The turntable was then moved slowly at a rate of 6° per sec. This procedure resulted in a smooth head movement such that the orientation of the head with respect to the environment was maintained. A plot of compensatory head angles as a function of the angle through which the turntable had moved revealed a linear function with a slope of .85 (a slope of unity indicating perfect fixation). As the angle of the head with respect to the prothorax approached zero, a small saccade occurred, followed then by typical OKN behavior.

The saccadic and smooth head movement mechanisms possess several differentiating characteristics. (1) The slope of the function relating stimulus angle to head angle is greater for smooth than for saccadic head movements. (2) Saccadic head orientations can be obtained by movements of a small target, whereas smooth head movements can occur in the total absence of any small "prey-like" stimulus. (3) The smooth movement system operates continuously and is presumably dependent upon visual feedback, whereas the saccadic system is intermittent and is independent of visual feedback.

Both smooth and saccadic head movements are utilized by mantids in a typical prey-catching sequence. Usually an appropriate prey insect will elicit an initial saccade. If the deviation of the prey from the prothorax is relatively large, then the mantis orients the entire body toward the stimulus. Movements of the body are accompanied by smooth movements of the head with respect to the body which result in the maintenance of the target image on the anterior ("foveal") region of the two eyes. The net result is that prior to prey capture both the head and prothorax are approximately aligned with the prey object. These findings suggest that two distinct neurologic mechanisms controlling head movements are required in orienting to prey, preceeding successful prey capture.

992 REGULATION OF FEEDING BEHAVIOR IN THE BLOWFLY: SPREADING OF THE LABELLAR LOBES. Gerald S. Pollack* (SPON: D. Haubrich). Dept. of Biology, Princeton University, Princeton, New Jersey 08540.

The feeding reaction of the blowfly consists of three distinct motor acts: extension of the proboscis, spreading of the labellar lobes, and sucking. Previous studies focused primarily on the control of proboscis extension and the amount of food imbibed. These studies indicated that feeding behavior is regulated by excitatory and inhibitory inputs from external chemoreceptors, which assess the palatability of food, and by inhibitory input from internal stretch receptors which monitor the presence of food in the gut. The present study investigates the regulation of lobe spreading by chemosensory input and internal state. Extracellular recording from the muscles which spread the lobes, the retractors of the furca (RF),was used to quantitatively assay lobe spreading.

Behavioral and electrophysiological experiments indicated that appropriate chemical stimulation of labellar taste hairs was necessary to elicit lobe spreading.

Simultaneous recordings from the RF and the muscles which accomplish sucking revealed that active lobe spreading was important to meal initiation, but was not necessary for continued imbibition.

Recordings of chemosensory and motor responses to sucrose stimulation of labellar taste hairs indicated that temporal summation between sugar receptor spikes was necessary to elicit motor output to the RF. A response decrement, localized within the central nervous system, and occurring independently for different labellar hairs, may participate in the termination of motor output.

Motor responses were more frequent and more intense when either the sucrose concentration of the stimulus or the number of hairs stimulated was increased. Therefore, lobe spreading is modulated by the intensity of sugar receptor input.

The role of satiety in determining motor output was examined by recording motor responses to sucrose stimulation before and after feeding. Feeding caused decreases in the probability and intensity of motor responses, but did not alter chemosensory responses. Previous studies have shown that stretch receptors which monitor the presence of food in the foregut and crop inhibit feeding behavior. The role of these receptors in the inhibition of lobe spreading was examined by cutting the nerves which carry their axons to the central nervous system. In operated flies, feeding did not affect motor responses to sucrose stimulation. Lobe spreading thus appears to be inhibited by activity in these stretch receptors.

Lobe spreading to labellar stimulation thus presents the fly with a second opportunity to decide whether or not to feed (the first decision being whether or not to extend the proboscis to tarsal stimulation). Labellar chemosensory input is likely to provide a more accurate assessment of the suitability of potential food. The existence of this second feeding decision may therefore be funtionally advantageous.

993 PLASTICITY OF FOOD-FINDING BEHAVIOR IN THE LAND SNAIL. Roger P. Croll* and Ronald Chase. Dept. Biology, McGill Univ., Montreal, Quebec.

Achatina fulica is a "giant" snail, indigenous to East Africa, and highly suited to neurobiological study. Snails were individually tested in a Y-maze olfactometer for their ability to discriminate between a food odor and an unodorized airstream or between two different food odors. Lesion studies showed that the behavior is mediated by the posterior (optic) tentacles. Under optimal conditions, snails choose an airstream containing lettuce odor over an unodorized airstream with 85-95% accuracy. Performance at this level is dependent upon: 1) A previous monophagous diet of lettuce for a period of at least several weeks, and 2) A period of starvation lasting at least 9 days just prior to testing. To measure the retention of food odor memories, two groups of snails were fed either cucumber or carrot for 86 days. The animals were later tested in the olfactometer at two week intervals with cucumber odor in one airstream and carrot odor in the other. During this period snails in both groups ate lettuce when not being starved for subsequent testing. The animals oriented preferentially to the odor of that food to which they had been conditioned, and the group preferences persisted for at least 120 days. Control experiments showed that the method of repeated testing did not significantly affect the results. The duration of the memory was confirmed by demonstrating that after 120 days the experimental animals could re-learn the cucumber and carrot odor preferences faster than naive animals could learn the preferences de novo. We have evidence that the reported olfactometer behavior represents food-seeking rather than "home"-seeking behavior. However, other preliminary experiments indicate that the findings may be specific to a distant orientation to food sources by olfaction, and that a separate system(s) may regulate food selection based on tactile or gustatory cues.

994 A COMPARISON OF THE EFFECTS OF MIDBRAIN MUTING LESIONS AND OCCIPITOMESENCEPHALIC TRACT LESIONS ON THE VOCALIZATIONS AND OTHER BEHAVIOR OF DOMESTIC CHICKS. N. C. de Lanerolle* and R.E. Phillips. Dept. of Anim. Sci., Univ. of Minnesota, St.Paul., MN 55108.

Based on earlier work we predicted that midbrain muting lesions and occipitomesencephalic tract section would both alter, in the same direction, the behavioral measures mentioned below. Groups of domestic chicks 1) with bilateral midbrain muting lesions (ML), 2) with knife cuts aimed at the occipitomesencephalic tract (OM), and 3) sham operated (C) were tested in situations that readily elicit vocalizations - 1) in the open field, 2) feeding after 4 hr food deprivation, and 3) before and after the presentation of a novel object. In the open field test C peeped significantly more than did either OM or ML groups, and OM chicks peeped more than did ML birds. The peeps produced by C were also louder than those produced by OM birds. Both ML and OM birds made fewer scanning head movements, while only OM birds made more comfort movements. During feeding, ML birds were nearly silent while C and OM birds produced twitters, and the number of twitters produced by OM and C chicks was not significantly different. ML but not OM lesions reduced pecks at food. OM birds pecked more and looked at the novel object longer than either C or ML chicks. These results indicate that changes in behavior resulting from both lesions occur in the predicted direction in the open field test, but not in the feeding test. These differences will be discussed.

995 PREDICTION OF GIANT AXON VOLTAGE CLAMP RESULTS ACCORDING TO GOMPERTZ KINETICS. Dexter M. Easton. Dept. Biological Sci. The Florida State University, Tallahassee, Fl. 32306.

The Gompertz growth equation is an exponentiated exponential function suitable for describing a wide variety of asymmetric, sigmoid, saturating relationships. The time course of conductance change of K^+ during voltage clamp in squid giant axon (Hodgkin and Huxley, 1952, J. Physiol. 117, 500) is well fit by the growth equation $g_K=\bar{g}_K exp\text{-}b exp\text{-}k_K t$, while the conductance to Na^+ follows the first derivative of a similar equation, i.e. $g_{Na}=Y_{Na} b k_{Na} exp\text{-}k_{Na} t$, where $Y_{Na}=\bar{Y}_{Na} exp\text{-}b exp\text{-}k_{Na} t$. In the equations b serves the same purpose as the exponent of m or n in the Hodgkin-Huxley equations, i.e. it sets the delay of the curves on the time axis. The specifying rate constants, k_K and k_{Na} are directly proportional to the membrane potential, and the maxima $\bar{g}_K$ and $\bar{Y}_{Na}$ bear sigmoidal (Gompertzian) relations to the voltage step. The equations are proposed as an alternative to the Hodgkin-Huxley equations, compared to which they are perhaps simpler, inasmuch as the rate constants of conductance change are set directly by the voltage step rather than through intermediate m,n,h processes. (Aided by Psychobiology Program, F.S.U.).

996 MAUTHNER-INITIATED STARTLE RESPONSE IN TELEOST FISH. R. C. Eaton, R. A. Bombardieri and D. L. Meyer. Dept. of Neurosciences., Sch. Med., UCSD, La Jolla, CA. 92093.

A characteristic behavior, which we call the "Mauthner-initiated startle response", was recorded and quantitatively analysed with high speed cinematography (200 frames/sec) after vibrational stimulation in 11 of 13 teleost species which possess Mauthner cells. As shown in Fig. 1, this behavior has: 1) an initial phase, the "fast-body-bend", lasting about 20 msec and consisting of a stereotyped displacement of the head and tail to one side and 2) a second phase, the "return-flip", consisting of a non-stereotyped flip of the tail to the opposite side. This startle behavior was also elicited by a visual stimulus in goldfish. We conclude that the fast-body-bend is the direct result of activation of one Mauthner cell, and we suggest that the return-flip may involve other hindbrain cells, likely postsynaptic to the Mauthner cell, though other mechanisms cannot be excluded.

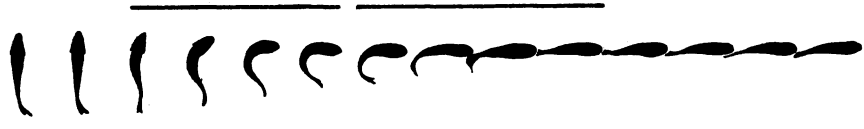

Fig. 1. Mauthner-initiated startle response of goldfish. Dorsal view silhouettes at 5-msec intervals. Each frame displaced to the right a constant distance. Stimulus occurred in the second frame from left.

997 ELECTRORECEPTIVE FEEDBACK IDENTIFICATION IN MORMYRID AND GYMNOTOID FISH
W.F.Heiligenberg, A 004 UCSD La Jolla, Cal. 92093

Electric fish assess their environment by monitoring electroreceptive feedback associated with their electric organ discharges (EODs). For optimal "electrolocation" performance animals have to distinguish between true feedback and sensations caused by EODs of other fish. Electric fish species which fire their electric organ in short pulses ("pulse-species") are most vulnerable to foreign pulses coinciding with or briefly preceding their own EODs whereas noncoincident pulses of comparable intensity do not impair electrolocation performance. These species thus are able to identify feedback from their own EODs and to discard sensations due to foreign pulses. Evidence is given that mormyrid and gymnotoid pulse-species found different solutions for this problem in the course of their convergent evolution: The former may gate electroreceptive input by corollary discharges of their electric organ pacemaker, the latter may gate electroreceptive input by signals of a set of specialized high threshold receptors only responding to the animal's own EOD (see J.Bastian's presentation). Therefore electrolocation in gymnotoid pulse species should suffer if noncoincident stimulus pulses of sufficient intensity are presented whereas mormyrids should be immune to this treatment. Preliminary behavioral experiments support this prediction.

998 REACTIONS OF FREE-FLYING GREEN LACEWINGS IN THE PRESENCE OF A HUNTING BAT. Lee A. Miller and Jens Olesen+. Institute of Biology, Odense University, Odense, Denmark, DK-5000.

We studied the reactions of free-flying green lacewings (Chrysopa carnea,Stephens) in the presence of a trained bat (Pipistrellus pipistrellus,L.) to demonstrate avoidance behavior predicted by Miller (J.Insect Physiol.21:205,1975) and to provide a behavioral foundation for neurobiological interpretations. A total of 80 freshly caught insects were used, some more than once, in 5 experiments with about 30 releases per experiment. Of the total number of releases about 120 insects were in range and in the bat's flight path. Voice notes of the results along with echolocating cries from the bat were recorded on a portable detector. The bat used "searching" cries with a fairly low repetition rate for the most part, and high cry repetition rates during detection and interception of prey. About 85% of the insects ceased their flight, folded the wings, and dropped passively in response to searching cries. In these cases the insects detected the bat first, thus avoiding predation. In about 12% of the interactions the bat detected the insects first, but only 3% of the detected insects were actually caught and eaten. We could not determine how the majority of detected insects were able to escape predation, but high cry repetition rates may have triggered a different type of evasive behavior. The flight cessation response of green lacewings to searching cries of a bat confirms earlier physiological studies on restrained insects. However,the possibility of "last chance" evasive maneuvers remains open.

999 ACOUSTIC PROPERTIES OF SINGLE NEURONS IN AGRANULAR FRONTAL CORTEX OF SQUIRREL MONKEYS. J. D. Newman, D. Symmes and G. E. Alexander. NIH, Bethesda, MD. 20014.

Click-evoked potentials have been recorded from the dorsolateral cortical surface between the central and arcuate sulci of partially decerebrate or chloralose-anesthetized squirrel monkeys (K. Bignall and P. Singer, Exp. Neurol. 18:300, 1967). The present study is an investigation of the acoustic properties of this region using microelectrodes and awake monkeys, and is an extension of a similar study concentrated on prefrontal cortex (J. Newman and D. Lindsley, Exp. Brain Res., in press). Clicks, white noise bursts, tone bursts, and taped recorded species - specific vocalizations were used as test stimuli. We recorded prominent click-evoked potentials over the are investigated. Polarity reversal as the recording electrode penetrated the cortex, and assessment of potentials contributed by the reference site (bone overlying occipital cortex), suggest that auditory evoked activity was locally generated. About 50% of the units tested exhibited activity changes time locked to acoustic input. The most effective stimuli were clicks, noise bursts and certain noisy (containing a wide frequency spectrum) vocalizations. Response patterns were similar between units, and for the same unit to different effective stimuli. Responses were characteristically brief ("on") and labile. These unit data are therefore substantially similar to those obtained from prefrontal cortex except for the greater percentage of acoustically activated units in the present study.

1000 EFFECT OF INTERRUPTION OF NORADRENERGIC FIBER SYSTEMS ON THE ONSET OF MATERNAL BEHAVIOR IN THE FEMALE RAT. Marianne K. Steele, David L. Rowland and Howard Moltz*. University of Chicago, Dept. of Beh. Sci., Chicago, Ill. 60637.

Primiparous female rats, 6 to 10 days post coitum, received bilateral electrolytic lesions aimed at the ascending noradrenergic fiber systems in the far-lateral hypothalamus. Those animals which, as a result of the lesioning, sustained a significant loss of hypothalamic norepinephrine (NE) failed to initiate maternal behavior during 13 days of testing postpartum; in contrast, animals failing to sustain a loss of hypothalamic NE were fully maternal. Levels of cortical NE and serotonin (5-HT), as well as hypothalamic 5-HT, did not differ between groups.

In a second study, lesions aimed at the dorsal NE ascending bundle were placed within the brainstem of primiparous female rats. In these animals, a deficit in the onset of maternal behavior was correlated with a profound loss of telencephalic NE, while hypothalamic NE levels were unaffected. The significance of these data for a hypothalamic-limbic NE system underlying the initiation of maternal behavior will be discussed.

1001 COMPARISON OF TEMPORAL AUDITORY AREAS WITH RESPECT TO RECEPTION OF SPECIES SPECIFIC SOUNDS. D. Symmes, J. D. Newman and G. E. Alexander. NIH, Bethesda, MD. 20014.

The use of species-specific vocalizations as test stimuli in studies of characteristics of neurons in the auditory system of awake squirrel monkeys has now been extended to cortical regions which are clearly secondary in their organization (are reciprocally connected to both auditory and nonauditory subcortical (structures). The present report summarizes our findings on a sample of 175 units lying on the lateral surface of the superior temporal gyrus within an area bounded dorsally by a line 2-3 mm below the sylvian fissure, auteriorly by the temporal pole, ventrally by the superior temporal sulcus, and posteriorly by an imaginary extension of the central sulcus. These units, referred to as "secondary", are compared with several previous samples of "primary" units isolated in or near the supratemporal plane in our laboratory (see Newman and Wolberg, Brain Res., 1973, 54:287-304, for review and original data).

The differences between the areas studied are both quantitative and qualitative, and are reasonably consistent with a hypothesized hierarchically organized cortical processor containing units specialized for detection of significant sounds. Cells responsive to some form of acoustic stimulation account for 73% of the total in secondary areas compared to 90% or more in primary areas. Cells responsive exclusively to vocalizations are equally rare, but "predictable" cells (similar response to vocalizations as to clicks, noise, and tones) are 3 to 4 times more common in primary areas. Responses closely linked to the energy envelope of the stimulus do not occur in secondary areas. Selectivity, defined as differential response to vocalizations sharing acoustical features, appears to be 2 or 3 times greater in secondary cortex. No subregion containing an unusually high proportion of cells specialized for vocalizations was found, nor were hemispheric differences observed.

1002 SPIDER WEB STRATEGIES: COMPARISON OF STRUCTURE AND CONSTRUCTION IN VARIOUS SPECIES. Peter N. Witt and J. Wesley Burgess, N. C. Mental Health Research, Box 7532, Raleigh, N. C. 27611

Innate patterns of behavior determine the ways in which spiders distribute silk; materials and energy are expended in exchange for food and shelter. Orb web builders (i.e. Araneus diadematus) live singly in a daily renewed geometric structure. Recycling of silk and modular design constitute economical ways to build large traps for flying prey. The webs aid in transmission of vibratory and chemical cues. Body size of the trapper is adjusted to prey size. — Social spiders (i.e. Mallos gregalis) live many thousand together in permanent structures of irregular three-dimensional design. It can be shown that vibrational and chemical cues aid them in distinguishing between prey and conspecifics. Many small individuals share one large prey. Silk production and distribution is probably predominantly carried out by subadults (R. Jackson).— In ontogenetic development of orb-web builders communal space-web building gives way to individual orb construction; simultaneously tolerant behavior toward conspecifics changes to universal predation. In other species of spiders (i.e. Metepeira spinnipes) both types of webs, orbs and space structures, are built side by side, each serving distinctly different functions: the orb is frequently renewed and traps prey for one individual, while the tangle persists over long periods of time and serves as substrate for living for many spiders at various ages. — Comparison shows that distinctly different patterns of inherited behavior can occur separately in different species, or together in one species, each simultaneously or at different stages of ontogenetic development. (Supported by NSF grant 0915).

Neuromuscular Junction

1003 A KINETIC DESCRIPTION OF ENDPLATE CURRENTS: AN EVALUATION OF THREE MODELS FOR THE ACTIONS OF ATROPINE AND SCOPOLAMINE. M. Adler*, E.X. Albuquerque, and F.J. Lebeda. Dept. Pharmacol. Exp. Ther., Sch. of Med., Univ. of Maryland, Baltimore, MD. 21201.

The decay phase of endplate currents (EPCs) in voltage-clamped frog sartorius muscle follows a single exponential function whose rate varies with membrane potential. Atropine increases the EPC decay rate and reduces its voltage sensitivity, but does not alter its exponential nature. Scopolamine transformed the EPC into a biphasic waveform consisting of a rapid initial current, followed by a slow terminal current. The magnitude of the terminal current was suppressed and its decay rate slowed by both high drug concentrations and hyperpolarized membrane potentials (Adler and Albuquerque, J. Pharmacol. Exp. Ther. 196:360, 1976). Heretofore, the best kinetic model to account for EPCs thus altered was a sequential reaction developed by Steinbach for local anesthetics (J. Gen. Physiol. 52:162, 1968).

$$\text{release} \longrightarrow \underset{\text{(diffusion \& hydrolysis)}}{\text{ACh}} + \text{R} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{AChR} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{AChR}^* \xrightarrow{D\ k_3} \text{AChR}^*\text{D} \xrightarrow{k_4} \text{ACh} + \text{R} + \text{D} \qquad (1)$$

In the model system, rapid reaction of acetylcholine (ACh) with receptors (R) results in the production of an inactive intermediate complex (AChR) which is followed by a slower voltage-sensitive conformational change leading to the formation of the conducting species (AChR*)(Magleby and Stevens, J. Physiol. 223:173, 1972). The decay phase of the normal EPC reflects the conformational relaxation of AChR* which is governed by the voltage-sensitive rate constant k_{-2}. According to reaction (1), when drug (D) is present, the EPC decays by an alternate route forming AChR*D. Depending on D, AChR*D may be inactive resulting in EPCs with rapid single exponential decays, or partially active producing biphasic decays. The reaction scheme was expressed as a set of first order differential equations whose solution by a digital computer yielded graphic outputs of model currents. The computer simulations revealed discrepancies with the experimental data, the most serious one being that neither the magnitude nor the decay rate of the terminal current was influenced by D. Therefore, an alternative scheme was formulated which preserved the essential features of (1) but introduced two modifications. First, AChR*D was assumed to be inactive regardless of the drug used. Second, the dissociation of AChR*D was assumed to occur by removal of D and restoration of AChR*, rather than by the unimolecular dissociation to ACh, R and D.

$$\text{release} \longrightarrow \underset{\text{(diffusion \& hydrolysis)}}{\text{ACh}} + \text{R} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{AChR} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{AChR}^* \underset{D\ k_{-3}}{\overset{D\ k_3}{\rightleftharpoons}} \text{AChR}^*\text{D} \qquad (2)$$

In reaction (2), the appearance of single or double exponential decay depends solely on k_{-3}. When k_{-3} is ~ 0, increases in D result in an acceleration of the EPC decay; as k_{-3} becomes appreciable an increase in D results in an acceleration of the initial component of the biphasic decay and a decrease in the magnitude and decay rate of the terminal component. The theoretical EPCs predicted by (2) agreed closely with experimental values. In addition to the sequential variants, a parallel model was considered in which D was assumed to interact with R prior to receptor activation. Atropine was assumed to transform R into a form which closes rapidly after activation; scopolamine was assumed to produce two altered receptor forms, one closing more rapidly, and the other more slowly than normal. The model EPCs were calculated as the sum of the parallel currents and had waveforms with rapid single or double exponential decay phases. The parallel reactions were also able to generate EPCs which agreed quantitatively with the experimental observations. (Supported in part by USPHS grant NS-12063 and the Paralyzed Veterans of America.)

1004 THE APPEARANCE OF ACETYLCHOLINE RECEPTORS AT NERVE-MUSCLE CONTACTS IN VITRO. Eric Frank* and Gerald D. Fischbach. Dept. of Pharmacology, Harvard Medical School, Boston, Mass. 02115.

At adult neuromuscular junctions, the subsynaptic membrane is extremely sensitive to acetylcholine (ACh). Chick muscle fibers innervated in vitro are also especially sensitive to ACh at sites of transmitter release. However, cultured muscle cells grown in the absence of nerve tissue also exhibit patches of high ACh sensitivity or "hot spots". It is possible that, like regenerating adult motor axons which reinnervate old endplates, embryonic axons seek out preexisting hot spots. Alternatively, ingrowing nerves might induce new patches of high ACh receptor density. To explore these alternatives, we have developed a rapid and precise method of "mapping" the distribution of receptors on single myotubes before, during and after innervation.

Small membrane patches are assayed by iontophoresing ACh from a high resistance micropipette. At each test point, the pipette is lowered until the maximum response is recorded (with an intracellular electrode) at which time the position of the pipette is recorded on a frame of 16mm film and the sensitivity is measured (mV depolarization/pC charge ejected) and recorded by a small on-line computer. In this manner 2-dimensional maps containing 50-200 points, separated by ca. 10 μm can be generated in 10-20 minutes and the same myotube can be assayed many times over a period of several days. The receptor distribution on uninnervated myotubes is stable; many hot spots remain in the same location and with the same sensitivity. Few new hot spots appear on uninnervated myotubes. Despite the stable distribution, hot spots reappear at the same loci following exposure to α-bungarotoxin which suggests that receptors within hot spots turn over rapidly.

Muscle fibers were innervated by thin cross-sections (explants) of 14-16 day embryonic spinal cord. These relatively old explants were used because distinctive large diameter nerve processes (not seen in younger explants) which are destined to innervate nearby muscle grow out from the ventral horns. Synapses can form within hours after nerve-muscle contact. In one case transmitter release was evoked (by extracellular stimulation in the presence of tetrodotoxin) from an active growth cone as it palpated the edge of a muscle fiber. We find that new receptor hot spots appear beneath neurites at newly formed synaptic contacts. Further experiments are needed to determine if contact of a preexisting hot spot is also a sufficient condition for synapse formation.

1005 RELATIONSHIP BETWEEN QUANTUM CONTENT AND MINIATURE ENDPLATE POTENTIAL FREQUENCY

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It would probably be acknowledged by most investigators that quanta giving rise to miniature emdplate potentials (MEPP) and to stimulus related endplate potentials (EPP) come from a common nerve terminal store. However, the relationship between MEPP frequency (MEPPF) and quantum content (m) of the endplate potential is an unknown. In a series of experiments with six agents or procedures on the frog sartorius nerve muscle preparation (1. Addition of zinc, 2. Replacement of Na with Li, 3. Doubling osmotic pressure with sucrose, 4. Tetanic Stimulation (50/sec and above), 5. Addition of Quabain, or 6. Addition of Black Widow spider venom) common factors were observed:

A. All methods caused an elevation of the MEPPF regardless of the calcium concentration (0-2 mM added Ca, and Ca free with EGTA);

B. With all methods it was possible to demonstrate facilitation of m (0.5 mM Ca);

C. With all methods the MEPPF attained high values with or without indirect stimulation (about 500 quanta per second, average maximum);

D. Concommittant with high MEPPF the endplate potential always failed. This failure did not correlate with loss of the nerve terminal spike;

E. Without removal of the facilitatory agent, terminals were eventually exhausted of quantal stores (mean, 400,000 quanta);

F. With removal of the facilitatory agent the EPP usually returned as MEPPF fell.

During all of the above experiments the total sustained quanta per second was never greater than about 500 per second (Total= MEPPF + m * stimulation frequency). It is suggested this occurs due to depletion of the releasable quantal store necessary for synchronous quantal release (m). The high MEPPF (500/sec) is due initially to depletion of the immediately releasable pool. MEPPF is then rate limited to the mobilization rate until the entire terminal is exhausted of quanta. Furthermore, it is suggested that the common factor of these six procedures that triggers asynchronous quantal release and the facilitated synchronous release with indirect neural stimulation is sodium entry into the nerve terminal with subsequent freeing of nerve terminal calcium stores. This intracellular calcium causes MEPP occurence and facillitated quantum content.

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1006 VOLTAGE-DEPENDENT EFFECTS OF HISTRIONICOTOXIN ON ENDPLATE CURRENTS. L.M. Masukawa*[1], E.X. Albuquerque[1], J. Daly*[2] and B. Witkop*[2]. Dept. Pharmacol. Exp. Ther.[1], Sch. Med., Univ. MD., Baltimore, MD. 21201 and Lab. Chemistry[2], NIH, Bethesda, MD. 20014.

Nonlinearity and hysteresis in the current-voltage (I-V) relationship of the endplate current (EPC) of the frog sartorius muscle were observed during voltage clamp experiments in the presence of 5 to 40 μM histrionicotoxin (HTX) under the following conditions. From a holding potential of -50 mV, the peak EPC amplitude was measured during command potentials which were continuously advanced in 10 mV steps (between -180 mV and +80 mV) after the EPC had been preceded by that potential for 3 sec. Acetylcholine activation of the receptor was eliminated as a coupling factor in the maintenance of nonlinearity and hysteresis from similar experiments in which short duration command potentials (20 msec) were made from the -50 mV holding potential at an interval of 3 sec. When EPCs occurred 10 msec after the start of the pulse, the I-V relationship was found to be linear and nonhysteretic.

The voltage-dependent rate of decrease of the peak EPC amplitude produced by HTX was monitored during 90 sec long command hyperpolarizing potentials by repeated activation of EPCs with an interval of 3 sec. The rate of decrease could be fitted by two exponential functions, one with a fast time constant ($t_{\frac{1}{2}} \simeq 25$ sec) and another with a slow time constant ($t_{\frac{1}{2}} \simeq 100$ sec). The first phase showed a voltage-dependence in which the rate increased with larger hyperpolarizing potentials. The rate of recovery of the EPC was very slow when the potential was returned to the holding potential of -50 mV.

At least two sites of action of HTX were deduced from the initial effect of the toxin on the EPC waveform at the holding potential of -50 mV. At a concentration of 20 μM the peak amplitude was reduced 50% and the rate of decay of the amplitude was increased by 60%. A change in the time course of the EPC has been taken to imply that the rate of opening and closing of the endplate channels has been altered. With digital computer simulations based on simple assumptions and in which HTX alters only the rate of closing of the EPC channels, it was shown that the change in the peak amplitude over a concentration range of HTX could not be fully explained by a single action but that a second site perhaps was curariform in nature.

Thus, the finding during the voltage-dependent decrease of the EPC amplitude that there was no further increase of the rate of EPC decay implied that a major effect of the hyperpolarizing potentials was on the interaction of HTX at this curariform-like site. The voltage-dependence of HTX action on the peak EPC amplitude appears to be the basis for the observed nonlinearity and hysteresis of the I-V relationship seen above. (Supported in part by USPHS grant NS-12063 and the Paralyzed Veterans of America.)

1007 LOCALIZATION OF ^{125}I-α-BUNGAROTOXIN BINDING AT FROG NEUROMUSCULAR JUNCTIONS. Julia A. Matthews-Bellinger* and Miriam M. Salpeter. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Recent availability of radioactive α-bungarotoxin (α-BTX) has provided a specific tool for the localization and quantification of the nicotinic acetylcholine receptor (AChR) at the neuromuscular junction (NMJ). Several studies of the mouse NMJ using electron microscope (EM) autoradiography have shown that this receptor is localized at very high concentrations (roughly 2-4×10^{4}sites/μ^2) on the post-junctional membrane adjacent to the axonal membrane (Fertuck and Salpeter, PNAS 71:1376, 1974: Porter and Barnard, J. Memb. Biol. 20:31, 1975; Fertuck and Salpeter, J. Cell Biol. 69:144, 1976).

Since most physiological studies of the endplate currents in response to ACh have been performed on frog muscle, we have extended the above studies to the frog in order to determine whether the combined physiological and molecular data allow us to make predictions regarding neuromuscular function. We therefore used ^{125}I-α-bungarotoxin-labeled NMJ's from Rana pipiens pectoris muscle to localize and quantify the AChR sites by EM autoradiography (with an autoradiographic resolution of 500Å). We found that, as in the mouse, the AChR sites are restricted to the regions of post-junctional membrane parallel to the axonal membrane and dipping only partway down the junctional folds. This membrane is characterized by an increased thickness and an electron dense staining, and constitutes ~50% of the total post-junctional membrane in this muscle. Since the frog NMJ has clearly defined and regularly spaced fold-interfold regions, and regions where Schwann cytoplasm is interposed between the pre- and post-junctional membranes, autoradiographic analysis of cross-sections through these different regions allowed us to demonstrate directly that the AChR sites are present only along the dense membrane at a concentration of 1.5-2.5×10^{4}sites/μ^2. Furthermore, the average AChR (α-BTX) site densities are the same in all regions, even where Schwann cell processes interpose between pre- and post-junctional membranes.

Given present knowledge regarding ACh activation of ion channels, it is probable that the rise time of the miniature endplate current (~150μsec) reflects the time during which the quantum of acetylcholine (ACh) is spreading to activate a progressively larger post-junctional area (Gage and McBurney, J. Physiol. 244:385, 1975). Recent physiological data indicate that 1-2×10^3 ion channels are opened per m.e.p.p. (Katz and Miledi, Nature 232:655, 1973; Anderson and Stevens, J. Physiol. 235:655, 1973). Assuming on the basis of Hill coefficients that at most three α-BTX binding sites are associated with one channel, one can calculate from our site density that 2000 channels (or 6000 α-BTX binding sites) could reside within 0.3-0.4μ^2. However, if ACh diffuses in the cleft with a rate constant of 10^{-5}cm^{2}sec^{-1} given by Eccles and Jaeger for free diffusion (Proc. R. Soc. Lond., Ser. B. 148:38, 1958), then a quantum of ACh would reach a post-junctional area of 0.4μ^2 within <50μsec and thus in a much shorter time than the characteristic m.e.p.c. rise time. Therefore, either the rise time of the miniature endplate current is not predominantly a function of ACh diffusion time, or not all the α-BTX binding sites are functional, or, as suggested by Gage and McBurney, the net rate of movement of ACh in the cleft of the neuromuscular junction is much slower than indicated by the free diffusion constant.

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1008 THE RELEASE-SPECIFIC COMPONENT OF PROTEINACEOUS EFFLUX FROM NEUROMUSCULAR PREPARATIONS. James R. Musick. Dept. Physiol., Sch. Med., University of Utah, Salt Lake City, UT. 84132.

Conditions which increase release of acetylcholine (ACh) from motor nerve endings in skeletal muscle also increase the concentration of proteinaceous material in the physiological solution bathing in vitro neuromuscular preparations, e.g., nerve stimulation of the paralyzed frog sartorius at 5/sec for 20 min produced increased Lowry (80 ng BSA equivalents/mg tissue) and Ninhydrin reactivity (8.5×10^{-8}M leucine equivalents/mg tissue). The known secretion of chromogranin proteins from sympathetic nerve endings supported the hypothesis that the release-specific proteinaceous material represented the soluble protein component of synaptic vesicles. However, some properties of this process are inconsistent with known properties of vesicular protein secretion: 1. The quantity of release-specific Lowry reactive material is estimated to be greater in mass (by up to 100-fold) than the synaptic vesicle population involved in quantal release. 2. Preliminary analysis of the release-specific proteinaceous material indicates a heterogeneous mixture, predominately composed of low molecular weight peptides and possibly amino acids. No known secretion of small peptides occurs at adrenergic synapses. 3. Release-specific polypeptide changes seen under certain conditions (by PAGE analysis) show elimination of a specific polypeptide of the control effluent and appearance of a polypeptide of higher electrophoretic mobility. Chromogranin secretion occurs without reported changes in control polypeptide components. 4. At low levels of evoked release in the rat diaphragm, ACh release could be measured (by pyrolysis-gas chromatography) in the absence of increased Lowry reactivity. Release-specific Lowry reactivity is non-linearly related to stimulation parameters; ca. 6,000 stimuli delivered at moderate rate (e.g., 5/sec) are necessary to detect significant increases in the bathing medium. Chromogranin and norepinephrine release are positively correlated over the range of release levels studied. The above difficulties in associating the phenomena under investigation with vesicular protein secretion can be resolved by postulating contribution of another process to the release-specific response. There is independent evidence suggesting an increase in extracellular proteolysis at the stimulated neuromuscular junction (Poberai, M., et. al., Neurobiol. 2:1, 1972). It is therefore proposed that at least a component of the release-specific proteinaceous material is the product of release-induced proteolysis, possibly by release of proteolytic enzyme(s) from nerve terminals. The non-linear relation between stimulation and release-specific Lowry reactivity in the rat diaphragm may indicate uptake of proteolysis products by pre- or postsynaptic elements; a process which must be saturated prior to detection of products in the extracellular medium.

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1009 EFFECT OF ETHANOL,OCTANOL AND PENTABARBITOL ON THE RATE OF DESENSITIZATION IN VOLTAGE-CLAMPED EEL ELECTROPLAQUE. Barry S. Pallotta*, George D. Webb* and Robert W. Sharp* (SPON: Richard E. Musty). Dept.Physiol. & Biophys., Univ. Vermont Col. Med., Burlington, VT 05401

Ethanol, octanol and sodium pentabarbitol significantly affect the rate of desensitization of Electrophorus electricus electroplaques. For all experiments with drugs the cells were exposed to the drug dissolved in physiological saline for 30 min., then drug in high K^+ saline for 10 min. The innervated membrane was then voltage-clamped to -75 mV, and, after about a minute for stabilization of current, 0.27 mM carbamylcholine (CCh) in the high K^+ saline with drug was added.

Cells exposed to 1M ethanol for 20 min. hyperpolarized by 1-3 mV and action potentials were reduced in magnitude and duration. After the 10 min. K^+-depolarization period and voltage-clamping to -75 mV, the rate of activation (determined from the current trace) by bath-applied 0.27 mM CCh was increased by the ethanol, but the extent of activation was unaffected. The half-time of desensitization ($t_{\frac{1}{2}}$) to CCh was 6.7 sec. ± 2.8 (mean±s.d.) compared to 18.1 sec. ± 6.0 for a paired control group (n=3, $p<0.05$,ANOV). The voltage sensitivity of the rate of desensitization was qualitatively unaffected, as we found that ethanol-treated cells desensitized faster at more negative potentials. Gage et al.(J.Physiol.244:409, 1975) have shown that ethanol prolongs the decay of MEPCs by increasing the duration of the elementary conductance event. Assuming that MEPC decay involves dipoles, they showed that if ethanol raises the membrane dielectric constant as expected, then the effects upon MEPC decay are quantitatively predicted. The rate constants for desensitization and EPC decay have opposite voltage sensitivity (Magazanik & Vyskocil, J.Physiol.210: 507,1970; Magleby & Stevens,J.Physiol.223:151, 1972); this implies that the presumed dipole moment changes associated with the two processes are opposite in direction. Therefore, acceleration of desensitization by ethanol would be expected.

1mM octanol caused a 1-3 mV hyperpolarization and blocked action potentials within 20 min. The rate of activation by CCh was slowed, with variable effects on magnitude. Desensitization $t_{\frac{1}{2}}$ was 58.1 sec. ± 3.2 compared to a paired control group $t_{\frac{1}{2}}$ of 21.1 sec. ± 4.7 (n=3,$p<0.05$,ANOV). Gage et al. (Life Sci.14:2277,1974) suggest that octanol shortens MEPC decay by disordering the lipid environment of the ACh-receptor protein. Since general anesthetics are known to enter the membrane lipid phase and lower membrane viscosity (Seeman, Pharmacol.Rev.24:583,1972), a slowing of desensitization by these drugs is expected. We found that 1.8 mM sodium pentabarbitol increases $t_{\frac{1}{2}}$ to 53.4 sec. ± 9.3 compared to a control $t_{\frac{1}{2}}$ of 39.4 sec. ± 7.9 (n=2,$p<0.05$,ANOV).

These results are consistent with a model for desensitization in which the conformational change or charge re-distribution of the ACh-receptor associated with activation raises the free energy of the membrane lipid-receptor protein system. The receptor's position within the membrane, or its conformation, would then change in order to minimize free energy once again and 'desensitization' would result. General anesthetics, which lower membrane viscosity, would raise membrane entropy and thus decrease the free energy. Since the receptor's hydrophilic interactions would be unaffected, the free energy difference between the pre- and post-activation conformations would be decreased. Since this free energy difference is the driving force for desensitization in this model, desensitization would take longer to develop.

Conversely, an increase in membrane viscosity might accelerate desensitization. Since Ca^{++} increases the viscosity of phospholipid monolayers (Shah & Schulman,Lipids 2:21,1967) it is possible that Ca^{++} increases the rate of desensitization through its effect on the lipid environment of the ACh-receptor. Supported by U.S.P.H.S. grant #ES00885.

1010 FREEZE-FRACTURE STUDY OF TRANSMITTER RELEASE AT NEUROMUSCULAR JUNCTIONS TREATED WITH BROWN SPIDER VENOM, BOTULINUM TOXIN, AND CATION IONOPHORES. D. W. Pumplin and T. S. Reese. NINCDS, NIH, Bethesda, Md. 20014.

Nerve terminals in frog cutaneous pectoris muscles treated with brown widow spider venom (BrWSV) or botulinum toxin (BotTX) were examined with the freeze-fracture technique. In terminals fixed during transmitter discharge, this technique reveals circular deformations in the presynaptic plasmalemma. Most are aligned within 75nm of ridges on the surface of the nerve terminal and represent synaptic vesicles fusing with the plasmalemma, but a few, typically larger and separate from the active zone (AZ), are thought to be coated invaginations where synaptic vesicle membrane is recovered (J. Neurocytol. 3:109). Therefore, the number and location of plasmalemmal deformations (PD) reflect primarily the rate and location of transmitter discharge. The number and distribution of PD on both sides of ridges, expressed as PD per running µm of ridge, were compared: after application of BrWSV, prepared by homogenizing the venom glands; 20 hours after injection of BotTX; and after electrical stimulation (ES) at 20Hz.

	at AZ	outside AZ	total PD	µm ridge
ES;2mM Ca	5.6	2.0	1696	224
BrWSV;2mM Ca	10.0	0.8	929	86
BrWSV;4mM Mg,1mM EGTA or 10mM Mg	3.2	3.2	1076	168
BotTX;ES;2mM Ca	0.07	0.04	22	98
BotTX;BrWSV;2mM Ca	1.6	0.8	174	72
BotTX;BrWSV;Mg,EGTA or 10 mM Mg	3.8	0.6	1330	353

Venom-induced PD are concentrated at the AZ in Ca-Ringer, but are spread over the presynaptic surface in a Mg-Ringer which blocks most of the discharge evoked by ES. Nerve terminals studied in serial thin sections after venom treatment in Mg-Ringer contain too few coated invaginations (<0.5 per AZ) to account for the PD outside the AZ. Therefore, synaptic vesicle fusion, as well as vesicle membrane recovery, can occur outside the AZ. The PD outside the AZ following ES presumably represent a mixture of synaptic vesicle discharge and recovery, although they represent primarily discharge in terminals treated with venom in Mg-Ringer. If venom increases the conductance of the presynaptic membrane to cations, then venom application in Ca-Ringer would produce a localized depolarization followed by Ca entry through the "normal" channel with the subsequent vesicle discharge occurring at the AZ. In Mg-Ringer, depolarization would not lead directly to release of transmitter, but entry of cations might cause an overall rise in the internal [Ca] due to displacement of Ca from internal stores. Such an overall rise in [Ca] could account for the more widespread appearance of PD in Mg-Ringer. The appearance of PD following ES is blocked by BotTX, indicating that this toxin inhibits vesicle discharge in addition to, or as a consequence of, the postulated inhibition of vesicle filling (Boroff et al, J. Physiol. 240:227). However, venom causes some vesicle discharge from BotTX-treated nerve terminals and, in Ca-Ringer, discharge at the AZ is selectively decreased. This result would be expected if the toxin inhibited depolarization-controlled Ca entry. However, in Mg-Ringer, discharge outside the AZ is decreased, suggesting that BotTX must have different or additional actions. The effects of the ionophores A-23187 (specific for divalent cations) and X-537A (transports both mono- and divalent cations) were compared to those of BrWSV. Both ionophores raise the frequency of mepps in normal muscles, but can induce few or no mepps in muscles treated with BotTX. These data suggest that the toxin may block a step in transmitter release which occurs after the entry of cations into the nerve terminal, and that the action of the venom may not depend entirely on increasing the permeability of the plasmalemma to cations.

1011 PHARMACOLOGY OF AN UNUSUAL MUSCLE CELL. M. Anderson and H. Mrose*. Dept. Biol. Sci., Smith College, Northampton, MA. 01060.

The myoepithelial cells of the proventriculus of the marine polychaete worm Syllis spongiphila consist of only 1 or 2 sarcomeres which may reach 40 μm in length. The cells are innervated by excitatory and inhibitory nerve fibers. We found that acetylcholine (ACh) mimics the excitatory transmitter and that the postsynaptic receptor sites appear to be nicotinic. Addition to the bath of ACh chloride (10^{-5}M - 10^{-3}M), carbamylcholine chloride (Carb, 5 x 10^{-4}M) or nicotine sulfate (5 x 10^{-4}M) elicited depolarizing responses, as did iontophoresis of ACh (10^{-1}M) or Carb (10^{-1} M). Addition of DL muscarine chloride (5 x 10^{-4}M) to the bath did not elicit comparable responses. d-Tubocurarine chloride (dTC, 10^{-5}M - 10^{-4}M) in the bath diminished in a dose-related manner depolarizations elicited by application of agonists or by indirect stimulation; dTC had no effect on hyperpolarizing responses. Atropine sulfate (10^{-6}M - 10^{-4}M) in the bath did not diminish responses to indirect stimulation. Eserine sulfate (5 x 10^{-4}M) potentiated the response to ACh added to the bath or applied iontophoretically.

When ACh was applied to the bath, the membrane potential depolarized to a peak and then fell to a plateau level 10-20 mV more positive than the resting level. Similarly, when a train of iontophoretic pulses of ACh was applied, the amplitudes of successive responses decreased to a plateau level. Both of these changes appear to be consequences of desensitization.

Finally, superimposed on the prolonged depolarization elicited by ACh added to the bath are smaller changes in potential that are abolished in 4-6 mM Mn++ or in Ca++-free solutions. These small changes in potential are likely to be from activity in neurons that receive cholinergic synapses. (Supported by USPHS Grant # RO1-NS12196).

1012 REDISTRIBUTION OF ACETYLCHOLINE RECEPTORS ON CULTURED MUSCLE CELLS. M. J. Anderson*, M. W. Cohen and E. Zorychta*. Dept. Physiol., McGill Univ., Montreal, Que.

Myotomal muscle cells from embryos of Xenopus laevis were cultured as a monolayer with and without neural tube cells. When neural tube cells were included many of the muscle cells became innervated: spontaneous twitching and contractions evoked by nerve stimulation were observed only in nerve-contacted muscle cells and this activity was abolished by curare and α-bungarotoxin.

By two days in culture non-innervated muscle cells were found to have one or more characteristic patches of ACh receptors as revealed by staining with fluorescent conjugates of α-bungarotoxin. Such patches were uncommon on innervated cells; instead the fluorescent stain was often confined to the path of nerve-muscle contact. Similar patterns were also observed within one day of adding neural tube cells to 2- and 3-day-old muscle cultures.

In a further series of experiments 3-day-old muscle cultures were stained with fluorescent toxin and maintained thereafter in native toxin in order to ensure that newly-synthesized receptors would not be stained. Neural tube cells were then added. When the cultures were examined 1-3 days later characteristic patterns of stain were observed at sites of nerve-muscle contact. In some examples the stain along the path of contact extended for greater distances than the patches associated with non-innervated cells, indicating that some of the receptors originally existed elsewhere on the muscle cell. Successive observations on individual muscle cells confirmed that receptors accumulate at sites of nerve-muscle contact and further revealed the formation of new receptor patches on non-innervated cells. Thus redistribution of ACh receptors occurs both spontaneously and as a result of nerve contact. (Supported by MRC of Canada).

1013 EFFECT OF ERYTHROSIN B ON NEUROMUSCULAR TRANSMISSION IN THE FROG. George J. Augustine, Jr.* and Herbert Levitan (SPON: W. Hodos). Dept. of Zoology, Univ. of Maryland, College Park, Md. 20742.

Erythrosin B is an anionic dye widely used as a food coloring (F D & C Red No. 3). Its approval for such use by federal agencies implies that it lacks significant biological activity. We have examined the sensitivity of a vertebrate neuromuscular junction to this food dye. The iliofibularis muscle of the frog (Rana pipiens) was isolated bathed in Ringers and the end plate region impaled with 1 or 2 glass microelectrodes filled with 3 M KCl. Spontaneous miniature end plate potentials (MEPPs) were monitored on a storage oscilloscope and/or pen recorder to determine amplitude and frequency of occurrence. The preparation was treated with the dye by perfusing the bathing chamber with Ringers containing Erythrosin B. At a concentration of 10^{-4} M, Erythrosin B caused an increase in MEPP frequency and mean amplitude. The frequency of MEPPs increased from a typical control level of .5/sec to a steady-state frequency of about 10/sec within 15 minutes. Onset of this effect occurs within 1 minute of initiation of dye perfusion. The mean amplitude of MEPPs, nominally .2 mV to .5 mV in normal Ringers, was doubled after 30 minutes of exposure to Erythrosin B. The resting membrane potential (RMP) increased in muscle fibres which had a pre-treatment RMP of 60 mV or less, but the dye caused little change in membrane potential when applied to fibres with RMPs greater than about 60 mV. The increase in RMP occurred within 1 minute of initiation of Erythrosin B perfusion. The increase in mean MEPP amplitude occurred whether or not the dye produced a change in the RMP. Since these results show that Erythrosin B has effects on both the pre- and postsynaptic members of the frog neuromuscular perhaps its use as an inocuous food additive should be reviewed. (Supported by NSF Grant GB-43141)

1014 **FURTHER STUDIES OF GLUCOCORTICOID EFFECTS ON MOTOR NERVE ENDING EXCITABILITY.** **Thomas Baker, Walter F. Riker, and Edward Hall*. Department of Pharmacology, Cornell University Medical College, 1300 York Avenue, New York, N.Y. 10021.**

It has been shown that short term, high, daily dose regimens of synthetic glucocorticoids enhance the excitability of peripheral motor axons in cat (Arch. Neurol. 32:688, 1975). This steroid effect involves the repetitive generating capacity of distal axon and its terminals. Thus the incidence of post-activation stimulus-bound repetition (SBR) of soleus motor axons in vivo is increased. As a result, the obligatory post-activation potentiation (PTP) is also enhanced. It has now been shown that the typical glucocorticoid augmentation of PTP can be caused by a single i.v. dose of methyl prednisolone (MP). However, the single dose study revealed a biphasic action of MP on motor nerve excitability: MP immediately and selectively suppressed SBR-PTP; recovery of SBR-PTP responsiveness required 4 hours; typical enhancement of SBR-PTP next emerged and peaked at 24 hours; this increase subsided over the next 72 hours. This MP time course also enabled the peripheral motor axon to be identified as the site of these actions, since the full biphasic effects develop in the sectioned axon. The glucocorticoid prototype hydrocortisone, when given as a single i.v. dose exhibits only the initial depressant phase. However, when given for a short term intramuscularly, in high daily doses, hydrocortisone causes the characteristic excitability augmentation in these motor nerves. Tetrahydrocortisol, the more polar metabolite of hydrocortisone, is entirely inactive, regardless of dose or the mode of administration. It is concluded that both natural and synthetic glucocorticoids directly enhance the excitability of motor nerve endings, following an initial transient depression.

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1015 INTERACTIONS BETWEEN NEOSTIGMINE AND HYDROCORTISONE AT THE MAMMALIAN NEUROMUSCULAR JUNCTION. George J. Blake* and Lynn Wecker* (SPON: D.M. Buxbaum). Northeastern Univ. Coll. Pharm., Boston, Mass,02115 and Vanderbilt Univ. Sch. Med., Nashville, TN,37232.

The effects of hydrocortisone (HC) and neostigmine (NEO) on neuromuscular function were studied to elucidate clinical findings of an adverse interaction in myasthenia gravis patients(Patten et al., Neurol. 24:442,1974).NEO alone produces a biphasic response in the indirectly stimulated rat phrenic nerve-diaphragm preparation,i.e.,an initial potentiation followed by a decreased stabilization phase.These responses were dose related,with maximal potentiation at 10^{-5}M (205% control) and total second phase blockade at 10^{-3}M.When preparations were pretreated with HC (0.1 mg/ml)which had no effect alone,the dose-response curve for potentiation was enhanced in a parallel manner,with 10^{-5}M NEO producing a 350% response.The second phase curve was altered by HC (0.1 mg/ml) in a non-parallel fashion,i.e.,there was increased response with low doses of NEO (10^{-6}-10^{-5}M) and increased blockade at higher doses (10^{-4}-10^{-3}M). The optimal dose of HC was 0.1-0.3 mg/ml.Higher doses (1.0 mg/ml) had either no effect or showed adverse interactions.

In 12 day denervated hemidiaphragm preparations,HC showed a slight increase in responses elicited by exogenous acetylcholine (ACh).Likewise, NEO alone also produced a left shift in the ACh dose-response curve.In combination, HC and NEO had an additive effect on responses to ACh.

These results,in light of recent studies that glucocorticoids increase choline uptake (Veldsema-Currie et al.,Eur.J. Pharmacol.,35:399,1976) leading to an increase in ACh synthesis (Arts and Oosterhuis,Neurol.,25: 1088,1975),support both a pre- and post-synaptic action of HC.The interaction between NEO and HC,however,is dose dependent.Proper dose combinations may be beneficial while adverse interactions may be due to improper dosing.

1016 CHARACTERIZATION OF A NICOTINIC CHOLINERGIC RECEPTOR IN 7-DAY CHICK EMBRYO HEART CELLS. S. Cox*, H.T. Hutchison*and R.L. Suddith* (Spon: D. Kunze). Div. of Neurochemistry, Marine Biomedical Institute and Department of Human Biological Chemistry & Genetics, Univ. Texas Med. Br., Galveston, Texas 77550.

Chick heart is innervated by inhibitory (cholinergic) and stimulatory (adrenergic) nerves. It has long been known that the receptors which mediate the cardioinhibitory and cardiostimulatant responses in intact chick hearts appear before morphologic innervation of the heart by the autonomic nerves. The inhibitory effects of acetylcholine (ACh) and responses to appropriate blocking agents were studied by 7-day chick embryo heart cells in culture. Seven-day chick embryo hearts were excised, minced and dissociated in 0.1% viokase. The cells were placed in tissue culture dishes in Ham's F-10 medium supplemented with 15% fetal calf serum, 5% horse serum and 1% penicillin-streptomycin-neomycin (PSN) and incubated for two days in a 37°C moist CO_2 incubator. Spontaneously beating aggregates were observed in these primary cultures and response to ACh was tested. Maximal reduction in beating rate to 50% of control was observed at 10^{-6}M ACh. This ACh response is effectively blocked by curare (10^{-8}M), but not altered by atropine (10^{-8}M). Thus, dissociated heart cells cultured from 7-day chick embryos apparently possess nicotinic receptors. Furthermore, these beating heart aggregates also respond to 10^{-6}M norepinephrine, thus suggesting the presence of adrenergic receptors. Supported by NIH grants NS 11354 & NS 11255 and a grant from the Muscular Dystrophy Associations of America. R.L.S. is a Muscular Dystrophy Research Fellow.

1017 FUNCTIONAL AND STRUCTURAL CORRELATES IN RAT MOTOR NERVE ENDINGS EARLY IN DENERVATION. Anna B. Drakontides and W.F. Riker. Dept. Anatomy, N.Y. Medical College, Valhalla, N.Y.; Dept. Pharmacology, Cornell University Medical College, N.Y., N.Y.

Mammalian motor nerve terminals (mnt), have been cited as initial loci of morphologic and functional changes following denervation. The present work sought to correlate mnt dysfunction and structural changes during early denervation. Using rats, unilateral cervical phrenicotomy in situ enabled later in vitro comparisons of paired innervated and denervated hemidiaphragm preparations. Thus indirect isometric twitch responses, PTP and edrophonium PDP, were compared at periods of 14-22 hours after nerve section. Following these studies, diaphragms were rapidly fixed for electron microscopy. Indirect twitch tensions of denervated diaphragms declined to 67% of control over the first 14 hours. Thereafter this response fell precipitously at a rate of 12.5%/hr. Transmission failure was complete at 22 hours. The incidences and magnitudes of PTP and edrophonium PDP also fell with time after denervation. The rate of PTP loss was greater than that of twitch. At 14 hours approximately one-third of PTP magnitude remained. Both PTP and PDP neared extinction at 18 hours. When twitch tensions had fallen to 50% of control (16-18 hrs.), ultrastructural degenerative changes were uniformly present. Mitochondrial swelling and disorganization of cristae were evident first. As functional losses progressed, further degenerative changes appeared: mitochondrial disruption, decreased vesicle density, vesicular clumping, vesicle disappearance, primary and secondary cleft widening, terminal retraction and Schwann cell replacement. At none of the times studied, were there any structural aberrations of either muscle or intramuscular nerves. The data show that early mnt dysfunction precedes or coincides with the earliest ultrastructural changes in mnt.
(Supported by PMAF and NINDCS-NB1447).

1018 USE-DEPENDENT CHANGES IN CONDUCTILE PROPERTIES OF CRAYFISH MOTOR AXON. Paul A. Fuchs and Peter A. Getting. Dept. of Bio. Sci., Stanford University, Stanford, Ca. 94305.

Extracellularly recorded action potentials of the claw opener excitor motoneuron undergo characteristic changes of waveshape and conduction velocity during repetitive firing. During a train of impulses at 40 Hz., the peak to peak amplitude of the extracellularly recorded action potential decreases by 5-10%, and the conduction velocity increases by 1-2% from the first to the second spike. The duration at half-amplitude increases by 5-15% from the first to the tenth spike in the train. These changes, which are positively correlated with frequency, can be prevented by perfusing the preparation with 5mM cobalt chloride, suggesting a dependence on Ca^{++}. Extracellular, nonspecific muscle responses recorded from nearby muscle fibers show facilitation which is similiarly sensitive to frequency and cobalt. These changes in action potential waveshape are more pronounced in tertiary and secondary branches than in the primary axon. Saline in which sodium had been replaced with choline, ($0\text{-}Na^{+}$), and 1 mM solutions of lanthanum chloride were used to test for the ionic basis of conduction in the axon. The distal segments of the axon were more resistant to the $0\text{-}Na^{+}$ solution, and more sensitive to lanthanum, than the proximal segments. The claw opener motor axon thus appears to use both calcium and sodium for spike propagation, with the relative contribution of calcium conductance increasing as one approaches the terminals of the axon. It is proposed that the changes in action potential waveshape seen with repetitive firing result from a use-dependent increase in calcium conductance, and that the associated increase in Ca^{++} influx may participate in facilitation.

1019 INTERACTION OF CHOLINERGIC AGONISTS WITH BATRACHOTOXIN AT THE ENDPLATE. D.L. Garrison*[1], E.X. Albuquerque[1], J.E. Warnick[1], J. Daly*[2] and B. Witkop*[2]. Dept. Pharmacol. Exp. Ther.[1], Sch. Med., Univ. of MD, Baltimore, MD, 21201 and Lab. of Chemistry[2], NIAMDD, NIH, Bethesda, MD. 20014.

The action of cholinergic agonists at endplate receptors of innervated frog sartorius and on extrajunctional receptors of chronically denervated rat soleus muscles was examined after exposure to batrachotoxin (BTX) and tetrodotoxin (TTX). Conventional microelectrode techniques were used for intracellular recordings and microiontophoretic application of drugs. Bath applied carbachol (CARB; 0.27-0.35 μM) depolarized the endplate of sartorius muscles from -93 ± 1.5 mV (mean ± SE) to -21 ± 2.1 mV in 15-30 sec followed by the disappearance of miniature endplate potentials (MEPPS). Within 30 min the membrane potential had returned to -57 ± 6.7 mV and MEPPS reappeared (J. Gen. Physiol. 56:218, 1970). When the sartorius was exposed to BTX (0.15-0.30 μM) the membrane depolarized from -93 ± 0.5 mV to -21 ± 3.6 mV and MEPPS disappeared. Subsequent exposure to BTX + TTX (3 μM) then resulted in membrane repolarization to -85 mV within 30 min. CARB (0.27-0.35 μM) was then applied together with BTX + TTX; the depolarization by CARB was reduced by nearly 80% of control depolarization but MEPPS were still absent. Similar results were observed with bath applied acetylcholine (ACh) during cholinesterase inhibition. The transient depolarization induced by microiontophoretic application of ACh at endplates of sartorius muscles was little affected by BTX whether or not TTX was present (J. Pharmacol. Exp. Ther. 176:511, 1971). BTX was likewise ineffective in blocking depolarization induced by ACh applied extrajunctionally on denervated soleus muscles and did not affect the ability of α-bungarotoxin to block neuromuscular transmission. The results suggest that alterations in CARB-induced depolarization at the endplate of skeletal muscle by BTX do not involve an interaction of BTX at the cholinergic receptor. (Supported by USPHS grant NS-12063.)

1020 NEUROMUSCULAR TRANSMISSION IN RED AND WHITE MUSCLES. Robert A. Gertler* and Norman Robbins (SPON: R.Plonsey). Depts. of Biomedical Engr. and Anat., Sch. Med., CWRU, Cleve., O. 44106.

Motor units of rat red muscle (soleus- SOL) fire tonically at low frequencies (~10Hz) while those innervating white muscles fire infrequent, short, high frequency bursts (~40Hz)(Fischbach and Robbins, J. Physiol.:201,305,1969). Experiments were done to determine whether transmission at neuromuscular junctions is optimal at frequencies similar to those experienced in vivo. Intracellular end-plate potentials (EPPs) were recorded from partially curarized SOL and white (extensor digitorum longus- EDL) muscles in Krebs solution. Quantum contents of the first EPP (M_1) and of the average EPP following early tetanic rundown (M) were calculated from trains of 40 and 200 EPPs evoked by stimuli at 5,10,20, and 40Hz. M_1, M and M/M_1 were significantly higher in EDL muscles at all frequencies ($P<.001$). M of EDL had a greater frequency dependence (decreasing from a mean of 110 at 5Hz to 56 at 40Hz) than did M of SOL (going from 52 at 5Hz to 37 at 40Hz). The possibility that these differences result from differences in normal motor unit activity was investigated by chronically stimulating the peripheral nerve innervating one EDL of 4 rats for 10-20 days at 10Hz for 10 sec. every 20 sec. for 4-8 hours daily. Contralateral EDLs were not stimulated. Although the succinic dehydrogenase staining pattern of EDL was converted to that of SOL, the frequency dependence of M was unchanged in chronically stimulated EDLs. (Supported by NIH Grants GM-01090 and RO1NS-09420).

1021 THE ACTION OF LIORESAL ON NEUROMUSCULAR TRANSMISSION IN THE RAT PHRENIC-NERVE DIAPHRAGM PREPARATION. M. Glavinović* (SPON: W.G. VanMeter). Dept. Anaesthesia Research, McGill University, Montreal.

Lioresal (β-(4 chlorophenyl)γ aminobutyric acid (Ciba-Geigy Ltd.), a clinically useful muscle relaxant, is believed to act principally in the spinal cord. The experiments described here were undertaken to explore the possibility of a significant, more peripheral action. Measurements of end-plate potentials (e.p.p.'s), end-plate currents, input impedances and resting potentials were carried out in diaphragms in vitro, at room temperature. The predominant effect of Lioresal (2.10^{-5}-2.10^{-4} M) was depression of neuromuscular transmission determined by recording e.p.p.'s in the presence of tubocurarine. This was associated with both pre- and post-synaptic changes. The most regular post-synaptic effect was an increase in conductance, seen at concentrations of Lioresal > 2.10^{-5} M. Resting potential changes were small (< 2 mV) and, if present, in the depolarizing direction, while ACh sensitivity appeared unchanged. Presynaptic effects were determined by measuring the quantal content of e.p.p.'s (in the presence of 19 mM Mg^{2+} or tubocurarine). At low concentrations of Lioresal (2.10^{-6}-2.10^{-5} M) the quantal content was increased, but at higher concentrations, it was depressed. It is concluded that the central relaxant action of Lioresal could be potentiated by more peripheral effects.

Supported by the Medical Research Council of Canada.

1022 THE EFFECT OF BLACK WIDOW SPIDER VENOM ON THE ACETYLCHOLINE CONTENT, ELECTRICAL ACTIVITY AND ULTRASTRUCTURE OF NEUROMUSCULAR JUNCTIONS IN MOUSE DIAPHRAGM. Alfredo Gorio*, Bruno Ceccarelli* and W.P. Hurlbut*. (SPON: F. Brink, Jr.) Rockefeller Univ. NY 10021 and The Institute of Pharmacology, Univ. of Milan, Milan, Italy.

When crude black widow spider venom is applied to mouse diaphragms in Kreb's solution at 37°C the frequency of miniature end-plate potentials (mepp) rapidly increases several hundred fold and then subsides. After an hour 89% of the junctions show no mepp's. Those junctions that remain active exhibit mepp's at frequencies ranging from 2-100/sec. None of these junctions responds to the application of 25 mM K^+ Kreb's.

Venom reduces the acetylcholine (Ach) contents (measured by bioassay with the guinea pig ileum) of diaphragms by about 60%. It increases the rate of release of Ach 3-4 fold during the first 15-30 minutes of application and then the rate subsides to control levels. After the diaphragm has been treated with venom for an hour the addition of 25 mM K^+ does not change the rate of release of Ach. Normally, 25 mM K^+ increases the rate of release 3-5 fold.

Our preliminary observations show that the venom depletes the nerve terminals of their vesicles.

These results indicate that Ach may occur in nerve terminals that are depleted of vesicles. However, it appears that this residual Ach cannot be released in either a quantized form, or in a non-quantized form, by high concentrations of K^+.

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We thank Dr. Andres Ronai, Montefiore Hospital, New York, for teaching us the ileum bioassay technique.

1023 TRIAMCINOLONE PRESERVATION OF MOTOR NERVE TERMINAL EXCITABILITY DURING EARLY DEGENERATION. Edward D. Hall*, Thomas Baker and Walter F. Riker,Jr. Dept. of Pharmacology, Cornell University Medical College, N.Y.,N.Y.10021.

Intensive treatment with glucocorticoids and primarily triamcinolone greatly increases the excitability of cat motor nerve (Arch. Neurol. 32: 688, 1975). In this work the effects of triamcinolone on motor nerve terminal function were examined during early degeneration. Cats were given triamcinolone (8mg/kg i.m.) daily for 7 days. On the last treatment day, a sciatic nerve was sectioned; 48 hours later an in vivo soleus nerve-muscle preparation tested motor nerve terminal excitability and neuromuscular transmission. In untreated cats, 48 hour denervation results in a syndrome of motor nerve terminal dysfunction (J. Gen. Physiol. 53:70, 1969): indirectly evoked twitch tension and the ability to maintain tetanic tension are reduced; the characteristic post-tetanic repetition of soleus motor nerve (PTR) and the dependent post-tetanic potentiation (PTP) are deficient. Although in triamcinolone-treated animals, the twitch tension loss was not prevented, the neurally generated PTP was strikingly maintained and enhanced 48 hours after motor nerve section showing an impressive glucocorticoid preservation of motor nerve terminal excitability. The number of motor nerve fibers generating PTR was increased from 17.5% in the untreated to 73.2% in the treated, ($p<0.01$). Triamcinolone treatment also improved the capacity of the terminals to carry repetitive stimulation and thus maintain indirect tetani. These findings disclose that certain steroids act on an axonal generator region, to increase its normal level of excitability and to preserve its excitable functions in the face of trophic deprivation. These results may be important therapeutically.

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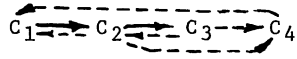
1024 A CLASS OF MODELS FOR TRANSMITTER KINETICS. K. N. Leibovic, Dept. Biophysical Sci., Sch. Med., SUNY, Buffalo, NY 14226.

Chemical transmission is characterized by a sequence of events in which stored transmitter is released, transported to specialized membrane structures (receptors) where channels or ionophores are activated and after performing this function the transmitter (generally inactivated) is returned again to storage. Numerous phenomena can be observed which have been interpreted as due to the mechanisms operating at various stages of transmission. Thus, at the neuromuscular junction one may observe, under suitable conditions, an initial potentiation and then a depression of the response which can be due to transmitter mobilization and depletion respectively. Under different conditions there may be receptor desensitization. To investigate factors such as these, influencing transmission and response a class of models has been analyzed. These may be illustrated by the following kinetic scheme.



C_1, C_2, C_3, C_4 are four possible transmitter states viz. stored, released, combined (with receptors) and inactivated transmitter respectively. The full arrows are obligatory, the dotted arrows alternative pathways, respectively. Transmitter is released upon stimulation and various rate parameters govern the different transitions. The stability of the systems and the effects of changes in the rate parameters on the qualitative behaviour of the systems have been investigated both analytically and by analog simulation.

1025 THE ACETYLCHOLINE CONCENTRATION IN THE SYNAPTIC CLEFT DURING NICOTINIC TRANSMISSION. H. A. Lester, D. D. Koblin* and R. E. Sheridan*. Division of Biology, California Institute of Technology, Pasadena, CA 91125

In voltage-clamped Electrophorus electroplaques, postsynaptic currents were measured in response to presynaptic nerve stimulation at a frequency (3/sec) leading to maximal facilitation of acetylcholine (ACh) release. Peak current-voltage curves were linear at negative potentials and showed a synaptic conductance of 140 mmho/cm^2 (3 cells). This equals the maximal conductance (150 mmho/cm^2, same cells) approached with steady exposure to high [agonist], at high negative potentials. We conclude that nerve-released ACh opens nearly all of the available ACh receptor channels. To measure the rate of channel opening, miniature postsynaptic currents were recorded focally; the growth time (20-80%) was 160 μsec. During this rising phase, the average receptor channel therefore undergoes the closed-to-open transition at a rate of $\delta = 1/(160\ \mu sec) = 6 \times 10^3\ sec^{-1}$. In voltage-jump relaxation studies with steady [ACh], we found $\delta = k_+[ACh]$, with $k_+ = 10^7\ M^{-1}sec^{-1}$ (15°). Values of δ exceeding $10^3\ sec^{-1}$ could not be observed with voltage jumps because δ desensitizes during steady exposure to high [ACh]. But assuming that we can extrapolate to higher δ during brief, nerve-released pulses of ACh, we find $[ACh] = k_+/\delta = 600\ \mu M$ during the rising phase of the miniature postsynaptic current. If the 10^4 ACh molecules in a vesicle diffuse in the cleft for 160 μsec, [ACh] within the rms distance is several hundred μM. All these findings are approximate and do not depend on a detailed molecular model for agonist-receptor-channel interaction.

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1026 β-BUNGAROTOXIN: THE RELATIONSHIP OF PHOSPHOLIPASE A ACTIVITY TO NEUROMUSCULAR TRANSMISSION. D.R. Livengood, R.S. Manalis*, M. Donlon*, G.S. Tobias* and W.G. Shain, Dept. of Neurobiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014 and Dept. of Physiology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 98760.

At least two different species of β-bungarotoxin (β-Bgt) have been isolated and studied (Kelly et al.,1975; Wernicke et al.,1975; and Donlon et al.,1975). We are proposing that the toxin may function by first binding to a proteinaceous moiety of the presynaptic membrane and then by phospholipase A (PLA) enzymatic activity. Subthreshold end-plate potentials (EPP) were recorded in frog neuromuscular preparation in 0.36 mM Ca^{++}, 2.9 mM Mg^{++} saline. EPP amplitude and membrane potential of muscle fibers were analyzed during treatment with purified β-Bgt or toxin treated by boiling at pH 8.6 to destroy PLA activity. Purified enzymatically active β-Bgt (0.2-2.0 μg/ml) causes a rapid decay in 5-10 min in EPP amplitude. This is frequently followed by a rebound of the EPP to levels in excess of control. This is in turn followed by a second decrease to zero (100-150 min). The toxin blocks the EPP but not iontophoretically applied ACh in a curarized preparation. Enzymatically inactivated β-Bgt (0.05-2.0 mg/ml) also causes a rapid decrease in amplitude of the EPP. No rebound of EPP activity has been seen with inactivated toxin. Loss of EPP amplitude with inactivated β-Bgt appears to be dose-dependent. We suggest that a molecule of β-Bgt has two functional components: 1) one a PLA active site and 2) a site that binds to the presynaptic membrane, probably at the Ca^{++} binding site and thereby blocks transmission.

1027 UNITARY EVOKED ENDPLATE POTENTIALS OF THE MOUSE DIAPHRAGM ARE COMPOSED OF SUBUNITS. F. Llados* and M. E. Kriebel* (SPON: D. Blank). Dept. of Physiology, SUNY Upstate Medical Center, Syracuse, N.Y. 13210.

Mouse hemidiaphragms were placed in a small bath mounted onto the stage of a compound microscope. Saline was aerated with 95% O_2 - 5% CO_2 and recirculated with a bubble lift. Muscle cells were penetrated under visual control. Nerve stimulation was accomplished with a suction electrode. After recording control MEPPs, Co^{++} was added (final concentration 3 - 4 mM) until EPPs were reduced in amplitude to the unitary evoked potentials (about 50% failures). Amplitude histograms of the unitary evoked potentials (4 - 6 mV) showed multiple integral peaks. The first peak (0.3 - 0.4 mV, depending on muscle cell diameter) corresponded to the first peak (s-MEPPs) of amplitude histograms of MEPPs (Kriebel & Gross, 1974, J. Gen. Physiol.). Amplitude histograms of MEPPs also showed integral multiple peaks which suggest that most MEPPs result from the synchronous release of several subunits (Kriebel, Llados & Matteson, in press J. Physiol.). The multiple peaks in the histograms of unitary evoked responses were integral multiples of the smallest, demonstrating that most unitary responses were composed of synchronously released subunits. Since the peak intervals of unitary evoked potential histograms were similar to those of MEPPs, the subunits of MEPPs and EPPs may be from the same store. (Support: PHS grant 5 R01 NS 11996-02)

1028 LEAD COMPETITIVELY BLOCKS EVOKED TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. R.S. Manalis, G.P. Cooper, and S.L. Pomeroy*. Departments of Physiology and Environmental Health, University of Cincinnati, Cincinnati, Ohio 45267.

Experiments were performed on superficial endplates in frog (Rana pipiens) sciatic nerve-sartorius muscle preparations which were mounted in a suitable chamber. Junctions were located visually using a compound microscope with a total magnification of 400x. Muscle contraction was prevented following nerve stimulation by bathing the preparations in high Mg^{++}/low Ca^{++} Ringer solution which, during the control periods, had the following composition (in mM): 111 NaCl; 2.0 KCl; .36 $CaCl_2$; 2.4-2.9 $MgCl_2$; 4.0 tris maleate. The temperature was 15°C; the pH was 7.1. Simultaneous extra- and intracellular records of averaged responses were obtained from the same endplate; this demonstrated that the endplate potential (EPP) (and the extracellularly recorded endplate current) decreased in the presence of lead while the nerve terminal spikes did not change. Thus, evoked transmitter release was not reduced by a mechanism involving a reduction of the amplitude of the presynaptic action potential. The amplitude of the mean EPP was plotted against the calcium concentration (range, 0.2-.66 mM) in the bathing medium on double logarithmic coordinates. The slope of the Ca^{++}- EPP relationship was close to four. In the presence of 1μM $PbCl_2$, the dose-response curves shifted to the right with no change in slope. Thus, lead acted via a competitive mechanism. Presumably, lead, like several other heavy metals, is more effective than is magnesium in competitively blocking the evoked release of transmitter.

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1029 EFFECT OF CALCIUM ON FREQUENCY OF MINIATURE END-PLATE POTENTIALS. Gary Matthews* and Warren O. Wickelgren (SPON: R.A. Lehman). Dept. of Physiology, Univ. of Colo. Sch. Med., Denver, CO, 80220.

The effect of Ca concentration on the frequency of miniature end-plate potentials (m.e.p.p.s) was studied at the frog neuromuscular junction. In Mg-free saline with normal [K] (2 mM), a small but reliable increase in m.e.p.p. frequency was observed as [Ca] was increased in steps from 0.5 to 6 mM. However, in solutions with raised [K] (5 and 11 mM), m.e.p.p. frequency increased with increasing [Ca] only in the range of 0.1 to about 2 mM, decreasing as [Ca] was increased further up to 10 mM. This nonmonotonic effect of [Ca] on m.e.p.p. frequency in partially depolarized preparations may be due to two opposing effects of [Ca]. Changing [Ca] (1) alters the driving force for Ca entry into nerve terminals and (2) alters the effective membrane potential of the nerve terminals by varying the screening of fixed negative charges on the outside surface of the nerve terminal. Since fixed negative charges can be assumed to exist in the vicinity of Ca channels and since Ca channels in the terminals are opened by depolarization, a reduction of external [Ca] would tend to open these channels while at the same time reducing the driving force for Ca. In saline with normal K (2 mM), the effect of [Ca] on the effective membrane potential may be unimportant since Ca-conductance in frog motor nerve terminals seems relatively insensitive to changes in membrane potential at values near rest, allowing the effect on driving force to dominate. However, in depolarized nerve terminals where Ca-channel opening is more sensitive to changes in membrane potential, reduction in driving force produced by lowering [Ca] may be more than compensated by the increased opening of Ca-channels resulting from the depolarizing effects of lowering [Ca]. (Supported by NIH grants NS-09661 and NS-50295).

1030 E. COLI ENDOTOXIN AS A NEUROTOXIN: ALTERED TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. Robert J. Person and Jaquelyn A. Kuhn*, Dept. Physiol. Biophysics, OU Health Sci. Ctr., Okla. City, OK 73190.

Endotoxin (ETX), a high molecular weight lipopolysaccharide derived from the cell wall of gastrointestinal bacilli, produces shock in intact animals which may be partially neurogenic in origin as a result of intense activation of the sympathoadrenal axis. Neurotoxic effects were observed with standard microelectrode techniques at the isolated frog neuromuscular junction exposed to E. coli ETX (Difco) at concentrations of 0.1 - 20 µg/ml bath. Endplate potential amplitude, recorded in either Mg^{2+} or tubocurarine paralyzed muscles, was rapidly depressed at 0.1-5 µg/ml and abolished at 10-20 µg/ml. Recovery of amplitude was variable and incomplete if washing commenced prior to block. Blockade could often be transiently relieved by high frequency stimuli. Miniature endplate potential (MEPP) frequency immediately increased upon exposure (5 - 40X control) then decreased to well below control rates (<0.1 Hz). The time course of the MEPP frequency effect varied as a function of ETX concentration and in vivo ETX potency, but mean peak frequencies were not altered. Peak frequency was reduced and the time course accelerated in low Ca^{2+} Ringer's. Terminal depolarization induced by 20 mM K^+ reduced ETX-elevated MEPP frequency but prolonged the time course. MEPP amplitude was significantly reduced simultaneous to the observation of subnormal release rates after prolonged ETX exposure. In experiments in which total quantal release could be estimated, total quanta did not exceed that expected after blockade of transmitter synthesis. ETX appears to exert significant presynaptic toxic effects similar to those produced by β-bungarotoxin and black widow spider venom.

(Supported by USPHS, NIH 5 SO1-RR05411)

1031 "NOISE" RELATED TO ELEMENTARY UNITS OF TRANSMITTER ACTION, DURING THE DECAY PHASE OF MINIATURE END-PLATE CURRENTS. D. M. J. Quastel and T. M. Linder.* Dept. of Pharmacology, The University of British Columbia, Vancouver, Canada.

The noise that occurs during the decay of miniature end-plate currents (mepcs) can theoretically yield information regarding the time course and size of the channels opened in the postsynaptic membrane by the action of endogenous transmitter. For example, if channels are of equal size and exponentially distributed in duration, for mepcs of average maximum amplitude an (where n is the mean number of channels and a is current per channel), the variance of mepc amplitude will be $a^2p(1-p)n$ plus terms depending on σ_n^2 and σ_p^2, at a time when the average number of channels remaining open is pn. Analysis of mepcs (prolonged in time course by ethanol) shows that such variance, i.e., "noise", does exist and its amplitude corresponds to unit events much the same as those produced by applied acetylcholine (ACh). A number of drugs (e.g., pentobarbital, atropine) split the decay phase of mepc into two components, one fast and the other slow. Kinetically the slow phase can be described either in terms of a prolonged life-time of a fraction of channels, or by oscillation of channels between "open" and "closed" conformations. The latter model predicts a much greater noise during the slow phase. Such noise is found experimentally: the amplitude of the unit is the same as with applied ACh and its time course appears to be the same as the fast phase of mepc decay.

1032 EFFECT OF CONCANAVALIN A ON BLACK WIDOW SPIDER VENOM ACTIVITY AT THE NEUROMUSCULAR JUNCTION. Lee L. Rubin*, Alfredo Gorio* and Alexander Mauro* (SPON: C. M. Connelly). Rockefeller Univ., New York, NY 10021.

Black widow spider venom produces massive release of transmitter at adult and at cultured neuromuscular junctions. In addition, spider venom causes profound morphological changes in particular regions of cultured neurons. Freeze-fracture and thin section analysis and preliminary biochemical experiments suggest an involvement of membrane lipids in these morphological alterations. We have now found that preincubation of cultured neuromuscular junctions with low concentrations (10 µg/ml to 100 µg/ml) of Concanavalin A (Con A) inhibits both the neuronal membrane disruption and the transmitter release normally produced by spider venom. Con A inhibition is partially or completely prevented by pretreatment with colchicine (10^{-4} M or 10^{-5} M) or with cytochalasin B (1 µg/ml to 10 µg/ml). Similarly, preincubation of frog neuromuscular junctions with higher concentrations (100 µg/ml to 300 µg/ml) of Con A blocks venom-induced transmitter release. Prior treatment with 10^{-6} M colchicine completely prevents the Con A effect. These results are formally analogous to those obtained from experiments on lymphocyte surface receptor capping. They suggest that redistribution of neuronal membrane components is a crucial step in spider venom action. Moreover, this membrane redistribution appears to be modulated in neurons, as in other cell types, by a microtubule-microfilament assembly. Neither Con A, nor colchicine, nor the combination has any striking effect on spontaneously occurring miniature end-plate potentials. The exact mode of action of spider venom remains to be clarified. Nonetheless, it appears from these results and from previous findings that the mechanism of venom-induced release is distinctly different from that of depolarization-induced release.

1033 PHOSPHOLIPASE ACTIVITY OF β-BUNGAROTOXIN IS RESPONSIBLE FOR ITS PRESYNAPTIC ACTION AT THE NEUROMUSCULAR JUNCTION. Peter N. Strong, John E. Heuser*, and Regis B. Kelly. Department of Biochemistry & Biophysics and Physiology, University of California, San Francisco 94143.

The presynaptic toxin, β-bungarotoxin (βTx) is a potent phospholipase (PLase). βTx shares many common enzymatic properties with other PLases (pancreatic, snake venom). The PLase activity of βTx is Ca^{++}-dependent (K=0.54 mM) and Mg^{++} cannot substitute. Sr^{++} is a competitive inhibitor (K=0.81 mM). Activity is stimulated by anionic surfactants (e.g. deoxycholate) and is maximized on attaining the critical micelle concentration of the surfactant/phospholipid micelles. Non-ionic surfactants (e.g. Triton X-100) inhibit PLase activity. βTx inhibits synaptic transmission only when Ca^{++} is present in the extracellular bathing fluid. When Ca^{++} is replaced by Sr^{++} (thereby blocking the toxin's PLase activity), synaptic transmission is normal but is no longer inhibited by βTx. Selective modification of βTx with N-bromosuccinimide or p-bromophenacyl bromide simultaneously eliminates both PLase activity and toxicity. Unlike βTx, normal PLases are not neurotoxic and do not block ACh release at equivalent enzyme concentrations. Similarly βTx lyses synaptosomes under conditions where other PLases are ineffective. Electron microscopy studies with βTx and peroxidase conjugated βTx, together with freeze-fracture data demonstrate that the toxin disrupts the presynaptic plasma membrane and enters the terminal. No evidence for βTx penetration is found in Sr^{++} media (Ca^{++} free). We suggest that the toxin inhibits release of ACh by selectively hydrolyzing phospholipids in nerve terminal membranes. Supported in part by NIH grant NS09878 (to R.B.K.) and a Postdoctoral Fellowship from the Muscular Dystrophy Association (to P.N.S.).

1034 CHOLINESTERASE INHIBITION AS A PRIMARY FACTOR IN PARAOXON-INDUCED MYOPATHY. Lynn Wecker* and Wolf-D. Dettbarn. Vanderbilt Univ. Sch. Med., Nashville, TN, 37232.

Paraoxon (PX),an organophosphorus cholinesterase (ChE) inhibitor, has been found to induce a grouped fiber necrosis in rat skeletal muscle.The pathology is associated with the motor end-plate region (Laskowski et al., Exp.Neurol.,47:290,1975)and may be neurally mediated.ChE activity in the rat hemidiaphragm preparation was studied to elucidate the role of the enzyme in the myopathic process. PX (.23 mg/kg,sc) produced an 85 % inhibition of neuromuscular ChE within 30 minutes and the enzyme remained significantly inhibited for the following 90 minutes.Administration of pralidoxime chloride (PAM, 60 mg/kg,ip),a phosphorylated ChE reactivator, at various time intervals after PX (10-120 minutes) increased ChE activity to 75 % controls.When administered 10 minutes after PX, PAM totally prevented lesion formation.At longer PX-PAM time intervals,there was a time related increase in muscle necrosis.If ChE inhibition proceeded for 2 hours prior to PAM administration,muscle necrosis (4.2 %) did not differ from PX control treated animals (4.7 %).Therefore, it appears that the myopathic process depends upon the degree and time course of ChE inhibition.

Denervation of hemidiaphragms totally prevented the necrosis induced by PX.ChE activity in denervated muscles decreased to 38 % of the contralateral side.It is well documented that this procedure significantly decreases end-plate ChE,while increasing non end-plate enzyme activity (Brzin and Majcen-Tkacev,J.Ce.. Biol.,19:349,1963).PX reduced the activity of the non end-plate ChE,but did not affect the low levels of end-plate enzyme.It appears that the myopathy is indeed neurally mediated and inhibition of end-plate ChE is a determining factor. (Supported by NIH research grant # NS12348 and a grant-in-aid from the MDAA,Inc.)

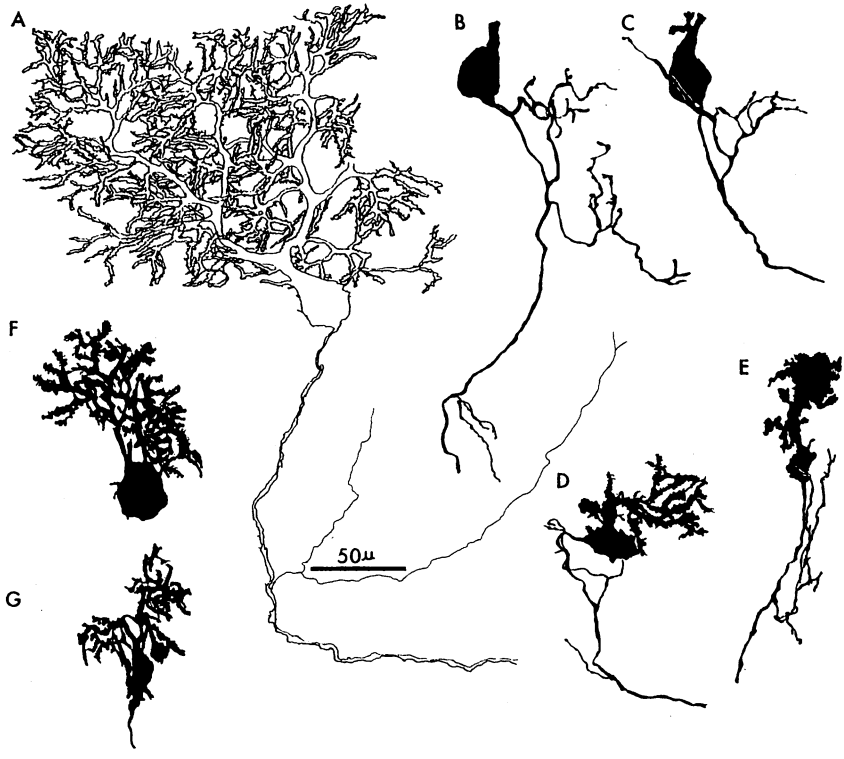
1035 EFFECTS OF GUANIDINE ON TRANSMITTER RELEASE AND NEURONAL EXCITABILITY. Warren O. Wickelgren and Gary Matthews*. Dept. of Physiology, Univ. of Colo. Sch. Med., Denver, CO, 80220.

Guanidine hydrochloride ($CH_5N_3 \cdot HCl$) applied to frog neuromuscular junctions in concentrations ranging from 0.1-0.5 mM produced a dose-dependent, reversible increase in the number of quanta released by presynaptic action potentials. No effect of guanidine on the postsynaptic membrane was observed. In saline containing 2 mM K, 0.3 mM guanidine had no effect on the frequency of spontaneous miniature end-plate potentials (m.e.p.p.s) but when the K concentration of the saline was raised above 3 mM, guanidine increased m.e.p.p. frequency. Guanidine also increased the excitability of motor nerve fibers, as evidenced by multiple firing to a single electrical stimulus and spontaneous action potentials. These effects on excitability were studied further using intracellular recording from giant reticulospinal axons (Muller axons) in the lamprey. Guanidine at concentrations similar to those used in the neuromuscular junction experiments increased excitability of these axons as demonstrated by a decreased threshold for action potential initiation, multiple firing to a single electrical stimulus, spontaneous membrane oscillations, and spontaneous action potentials. All of the effects of guanidine on excitability of Muller axons were mimicked by reducing the divalent cation concentration of the saline. It is suggested that guanidine (a monovalent cation) increases transmitter release and neuronal excitability by competing with divalent cations for binding sites in the vicinity of gated Na and Ca channels and, thereby, producing an effective membrane depolarization across these channels. (Supported by NIH Grants NS-09661 and NS 50295).

Neuropathology and Neuroimmunology

1036 A GOLGI ANALYSIS OF CEREBELLAR HYPOPLASIA PRODUCED BY LYMPHOCYTIC CHORIOMENINGITIS (LCM) VIRUS INFECTION IN NEONATAL RATS. David G. Amaral, John A. Foss, Andrew A. Monjan, and Manuel del Cerro. Center for Brain Research and Dept. of Psychology, Univ. of Rochester, Rochester, N.Y. and Dept. of Epidemiology, The Johns Hopkins University, Baltimore, Md.

LCM virus, when injected in 4-day old rats, produces an acute destructive necrosis of the cerebellum, starting approximately 4 days later. Rats injected on day 4 were sacrificed on day 14 and the brains were stained by the Golgi-Cox or rapid Golgi methods. By day 14, necrosis in the cerebellum was extensive and recognizable Purkyně (P) cells were observed only in the older lobules. In the figure below, a normal P cell [A] is shown which was drawn from a vehicle-injected littermate. No P cells in the virus injected animals had developed to this extent. Characteristically, the dendritic trees of P cells in LCM treated rats were markedly atrophic. The dendrites were distorted, often drooped, and generally contained large bulges of cytoplasm [D-G]. Apparently, as the P cell degenerated, the dendrites coalesced into one large mass of cytoplasm [E]. P cell axonal collaterals were more numerous and appeared to be hypertrophic[B-E]. Their extensive proximal collateralization also distinguished them from normal P cell collaterals. It is of interest that Ramon y Cajal observed similar modifications in P cells after undercutting cerebellar folia (see S. R. y Cajal, Degeneration and Regeneration, Vol. II, pp. 597-630). A developmental study is now in progress to determine whether the abnormalities described here in day 14 cerebellum are apparent before marked cytolysis is manifest.



1037 NEURONAL INVOLVEMENT IN PERIPHERAL NEUROPATHY IN DIABETIC MICE. Keith A. Carson, Edward H. Bossen* and Jacob S. Hanker. Dental Research Center and Neurobiology Program, UNC, Chapel Hill, NC. 27514 and Dept. of Pathology, Duke Univ. Medical Center, Durham, NC. 27710.

Previous studies in our laboratory (Hanker et al., J.Dent. Res. 55: 109, 1976) have demonstrated the appearance of a hind limb sensory ataxia in mice (C57BL/KsJ-db, The Jackson Laboratory) afflicted with an autosomal recessive disease resembling human diabetes mellitus. Microscopic studies revealed a proliferation of Schwann cells, many of which contained osmiophilic lamellar bodies. It has been suggested, however, that the Schwann cell hyperplasia and even the segmental demyelination frequently observed in human diabetes could be secondary to an insult to the neuron.

Because of the involvement of the hind limbs, it was of interest to compare sciatic nerve axons and lumbosacral spinal ganglion cells of ataxic diabetic mice with those of their unafflicted littermates. Mice 8-12 months of age were sacrificed by cardiac perfusion with a combination aldehyde fixative. Sciatic nerves and lumbosacral spinal ganglia were removed, immersed in the same fixative for 2 hours, osmicated and processed for electron microscopy.

Spinal ganglion cells frequently exhibited changes characteristic of the classical "axon reaction", such as displaced nucleus, focalization of the Golgi apparatus, and an increase in the number of free ribosomes. A more dramatic feature, however, was the large increase in osmiophilic lamellar bodies in the cytoplasm and even their appearance in nuclei of ganglion cells. Less frequently, large vesiculated osmiophilic bodies were observed in axons. In addition, there was evidence that some axons were undergoing degeneration.

The ultrastructural changes reminiscent of the "axon reaction" and suggesting axonal degeneration could be responsible for the neurological symptoms, because similar changes have been observed in mice afflicted with dystonia musculorum, an hereditary sensory neuropathy (Duchen et al., Brain 87: 367, 1964). The lipid inclusions in neurons, satellite and Schwann cells could be related to decreased lipolysis which has been observed in other tissues in these mice (Steinmetz et al., Diabetologia 5: 373, 1969). The decreased lipolysis in tissues of diabetic mice is probably due to a deficiency in adenyl cyclase activity (Kupiecki and Adams, Diabetologia 10: 633, 1974). The derangement in lipolysis could be responsible for the lipid accumulations in neurons as well as in Schwann cells of afflicted nerve in diabetic mice. Thus, our studies indicate that diabetic neuropathy could result from a disturbance in lipid metabolism of neurons as well as of Schwann cells — proposed by Bischoff (in Diabetes, Ostman, J. and R.D.G. Milner, eds. Amsterdam, Excerpta Medica Foundation, 1969).

This investigation was supported by NIH grants DE 02668, DE 00288, MH 14277, HL 15888 and RR 05333.

1038 ELECTROPHYSIOLOGIC EVALUATION IN MAN OF CENTRAL NERVOUS SYSTEM PATHWAYS FOLLOWING ACUTE HEAD TRAUMA. Richard P. Greenberg* and Donald P. Becker. (SPON: R. Sakalas). Div. Neurosurgery; Medical College of Virginia; Richmond, VA. 23298

It is difficult by clinical neurologic examination to evaluate, early in the hospital course, the location and extent of brain injury in patients with severe head trauma. We hypothesize that brain electrical activity measured by carefully performed, multi-modality averaged evoked potentials (a non-invasive technique) may be a more sensitive index of the metabolic and functional integrity of the central nervous system. Evoked potentials can be utilized to improve the accuracy of initial evaluation, management, and assessment of outcome in head injury patients.

Thirty patients from 4 to 65 years old, all unresponsive to verbal command on admission, had 46 studies performed. Each study utilized sixteen scalp electrodes distributed symmetrically over both hemispheres according to the International 10-20 system. Somatosensory visual and auditory evoked potentials, both near field and far field, were always recorded from each patient. Electroretinograms, VIIIth nerve action potentials, and peripheral nerve action potentials were done, when indicated, to verify integrity of peripheral receptors. The evoked potential data was correlated with serial clinical neurologic examinations, E.M.I. scans, angiography, ventriculography, operative and post-mortem findings when available.

In 80% of cases it was possible to correlate recovery of consciousness and presence or absence of residual neurologic deficits with evoked potential data collected within the first nine days. When severe focal or generalized evoked potential abnormalities appeared in the initial study there was often no improvement in either the electrical data or the patients conditions. From the study of evoked potentials it appears possible to separate patients with pure brain stem injuries from those with pure supratenorial injuries or mixed lesions.

1039 RETROGRADE AXONAL TRANSPORT OF HORSERADISH PEROXIDASE IN MOTOR NEURONS INNERVATING SLOW AND FAST MUSCLES IN NORMAL AND DYSTROPHIC CHICKENS.

Theodore Hoekman*, Mark DeSantis, and Visaka Limwongse*. Depts of Pharmacol and Anat., Georgetown Univ., Washington, D.C. 20007

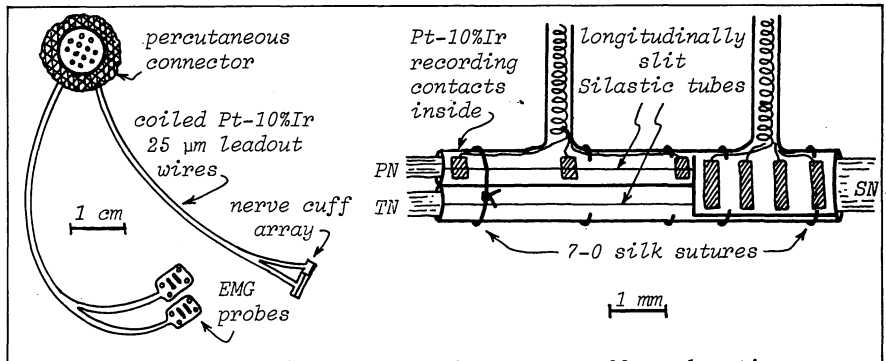
A reversed rapid axonal flow has been reported for motor and sensory neurons of normal chickens (Brain Res. 33: 560, 1971; 98: 291, 1975). We report here a comparison of the phenomenon in neurons innervating slow and fast muscles of normal and dystrophic birds. Normal (line 200) and dystrophic (line 304) New Hampshire chickens (1.1-1.6 kg) were anesthetized with pentobarbital and a 5% solution of horseradish peroxidase (HRP; Sigma, type VI) in 0.3 ml of saline was injected into the posterior latissimus dorsi (PLD) or anterior latissimus dorsi (ALD) muscle. After a survival time of 40 hours the animals were sacrificed by cardiac perfusion with an aldehyde fixative. Frozen sections of spinal cord segments 13-16 were incubated with 3,3'-diaminobenzidene (0.05%) and hydrogen peroxide (0.01%) in maleate buffer (pH 7.4). The sections were examined in the bright and darkfield of a light microscope for the presence of the granular reaction product in neuronal somata. The location and number of HRP labeled cells were recorded. Retrograde transport of HRP occurred in motor neurons innervating the PLD and ALD muscles of both normal and dystrophic chickens. HRP-containing motor neurons were located in the dorsolateral part of the ventral column of gray matter in segments 13-15. Counts of PLD or ALD neurons indicated there was no difference between normal and dystrophic birds in the number of labeled neurons. The number of labeled motor neurons after injections of ALD was consistently less (about 1/2) than after PLD injections. A greater percentage of ALD neurons was located in segment 14, whereas the greatest percentage of PLD neurons was in segment 15. No cells contained HRP reaction product on the side of the spinal cord contralateral to an injected side. The similarity between normal and dystrophic chickens in the number and location of HRP labeled motor neurons suggests that the integrity of the mechanisms for uptake and transport of HRP is maintained in the dystrophic chicken. The more rostral distribution of ALD motor neurons, as compared with those innervating PLD, corresponds to the different origins of the two muscles along the length of the body axis. The difference in the total counts of ALD and PLD labeled motor neurons may reflect an actual difference in the number of motor neurons innervating the two muscles, or a difference in uptake, rate of transport or degradation of HRP in neurons innervating the two muscles.

(Supported by USPHS grants NS 11177 and NS 11026.)

1040 *LONG-TERM PERIPHERAL NERVE AND MUSCLE RECORDINGS IN INTACT MICE.* *J.A. Hoffer, T.E. Milner* and R.B. Stein. Physiology Department, University of Alberta, Edmonton T6G 2H7, Canada.*

We have previously reported applications of our long-term recording methods from peripheral nerves of intact animals, using insulated cuff electrodes, to study rabbit locomotion (Hoffer et al., Soc. Neurosci. Abstr. 300, 1974; Hoffer and Marks, Neurosci. Abstr. 1, 244, 1975) and to model in cats possible methods of controlling prostheses in amputees (Stein et al., Can. J. Neurol. Sci. 2, 235, 1975). We present here results of long-term neural and EMG recordings from intact mice, which form part of a series of experiments designed to study the changes in activity in nerve and muscle that accompany progressive degeneration in murine muscular dystrophy. In five normal mice 5-10 weeks old we implanted electrode arrays (Fig. 1) consisting of peroneal (PN) and tibial (TN) nerve cuffs (300 μm I.D., 5 mm long), joined to a larger sciatic cuff (SN). The PN and SN cuffs each had three recording contacts inside. Bipolar EMG probes were sutured onto the muscles anterior tibialis (AT) and lateral gastrocnemius (LG). Cables were run under the skin and ended in a percutaneous connector. We monitored spontaneous voluntary activity and, under anesthesia, triphasic neural and biphasic EMG responses evoked by SN or PN stimulation, up to 10 weeks after implantation. 20-50 μV afferent unitary spikes of cutaneous, footpad and AT muscle receptors were resolvable. Phasic neural activity accompanied limb movements. The following changes in signal characteristics were observed: 1) electrode impedances typically rose gradually over the first weeks after implantation, probably due to connective tissue invasion of the cuffs. 2) AT EMG evoked by PN or SN stimulation decreased over the first few days, while conduction time increased, probably due to temporary blockade of some motor fibers following nerve dissection and cuff installation, but these effects reversed after the first week and EMG reached levels about twice as high as on the day of surgery. 3) LG EMG evoked by SN stimulation showed the same early changes as AT EMG, but in two mice a later recovery was not observed. This was attributed to the tighter fit of the cuff on the tibial nerve (300 μm thick) compared to the peroneal nerve (250 μm). Histology in one mouse after 5 weeks showed that the total number and size distribution of axons in the peroneal nerve were essentially similar to the contralateral control, while the tibial nerve showed a diminution in the numbers of large axons.

Thus, if cuffs are properly matched to nerve dimensions, and if care is taken to minimize nerve injury during surgery, chronic nerve recording from intact mice is possible. We are currently repeating these experiments in dystrophic mice and phenotypically normal siblings.



1a. Complete electrode array *1b. Nerve cuffs, schematic*

Figure 1

Supported by a Post-Doctoral Fellowship to JAH and a research grant to RBS from the Muscular Dystrophy Association of Canada.

1041 WALLERIAN DEGENERATION IN THE OPTIC NERVE OF THE NEWT: AN ULTRASTRUCTURAL STUDY. Stephen C. Johnson. Dept. Anat., College of Med., Univ. of Utah, Salt Lake City, Utah. 84132.

Wallerian degeneration in the perichasmatic portions of the optic nerve of the newt (Triturus pyrrhogaster) was studied at intervals ranging from 2 to 47 days following an extracranial crush-freeze lesion. Signs of autolytic unmyelinated axon degeneration are first apparent at 2 days with massive degeneration 4 days post-lesion. Initial stages of degeneration include loss of microtubules and neurofilaments and axoplasmic darkening, followed by disintegration of the plasma membrane and segmentation. Intracellular breakdown by reactive astrocytes begins at 4 days, and is complete by 10 days when signs of regenerating axons first appear.

The early stage of intraaxonal myelinated axon degeneration begins at 4 days and is characterized by a "light" pattern of axoplasmic degeneration and loss of microtubules and neurofilaments. "Dark" patterns of axoplasmic degeneration and axolemma disintegration characterize the intermediate and late stages which predominate after 4 days.

Involution and densification of the outer cytoplasmic loop of the myelin sheath is visible as early as 4 days and suggests early loss of cytoplasmic continuity with the parent oligodendrocyte. These changes in the outer loop preceed degenerative changes affecting the myelin sheath which involves a rapid process of unraveling and lamellar disintegration. Phagocytosis and intracellular breakdown of myelinated axons is efficiently carried out by microgliacytes and is well underway by 10 days and complete by 47 days.

Thus it appears that Wallerian degeneration in the optic nerve of the newt occurs more rapidly than that reported in mammals, and is a result of extensive autolytic axon and myelin degeneration followed by efficient removal and intracellular breakdown by glial cells. This rapid rate of degeneration may be an important factor favoring regeneration.

1042 MICRODETERMINATION OF EDEMA IN TRAUMATIC SPINAL CORD INJURY BY USE OF A SPECIFIC GRAVITY GRADIENT. R. S. Kagan*, J. L. Alderman, R. Moberg, J. L. Osterholm. Dept. Neurosurg., Thos. Jeff. Med. College, Phila., Pa. 19107.

Experimental spinal cord injury (SCI) results in a delayed hemorrhagic necrosis (HN) that originates in the central grey matter and spreads radially into the surrounding white matter after a delay of approximately 3-6 hours. The edema associated with SCI has been grossly quantitated, and although this event has been generally correlated with progressing HN (Yashon et al 1973, Griffiths et al 1974), the methods used to date have lacked the sensitivity required to quantify edema on a specific regional basis. This shortcoming was circumvented by coupling the specific gravity gradient column method of Lowry (1945) and modified by Nelson (1971) with a tissue punch technique. Edema was measured in samples weighing less than 1 mg., taken from several discrete regions of the cat spinal cord 1 hr. following a standard 500 gm. cm. injury at the thoracic - 8 level. Samples were punched from the posterior horns, anterior horns, anterior, posterior and lateral white columns from 1 mm. cross sections progressing longitudinally below, through and above the injury site. At the longitudinal center of the injury, the posterior horns exhibited an increase in tissue volume as water of 84% and the anterior horns exhibited an increase of 116%. Progressing rostrally and caudally from the injury, the edema was shown to steadily decrease approaching similarly treated control values at 2 mm. above and below the point of impact. The white matter was not significantly edematous at this time point, with the exception of the posterior white column (the region that comes into direct contact with the injuring force) which displayed a 64% increase in tissue water. This edema progressed 1 mm. above and below the injury center. It is concluded that this quick, simple inexpensive method can detect regional alterations in edema that have heretofore gone unnoticed due to a dilution effect induced by the use of large samples. By facilitating precise correlation of edema and necrosis, this method will help reveal the role of edema in spinal cord autodestruction, correct for possible artifactual influences of tissue swelling when biochemical determinations are to be made, be useful in assessing the reproducibility of inter-laboratory experimental injury and enable interactions of edema with other patho-physiologic sequelae to be determined in regions of active necrosis.

1043 TISSUE LACTATE ACCUMULATION (>15-20 umoles/g) AS CAUSE OF CEREBRAL EDEMA. Ronald E. Myers and Michio Yamaguchi. Laboratory of Perinatal Physiology, NINCDS, National Institutes of Health, Bethesda, MD. 20014

The distribution and extent of brain pathology produced by hypoxia, hypotension (Gamache, F.W. and Myers, R.E.: Arch Neurol 32:374, 1975), cyanide intoxication, carbon monoxide poisoning (Ginsberg, M.D. and Myers, R.E.: Arch Neurol 30:209, 1974), and other states characterized by retarded tissue oxygen utilization correlate poorly with severity of insult. For example, monkeys show variable relations between the oxygen tension of arterial blood during hypoxia and later brain pathology even when blood pressure changes are taken into account. Animals in which blood pO2 is maintained as low as 17 mm Hg for 25 minutes may fail to show brain damage while others in which it is controlled as high as 22 mm Hg may later develop brain edema and widespread injury to structures in the hemispheres. Similarly, though animals in which blood pressure is lowered to 28-30 mm Hg for 30 minutes generally develop brain edema and cerebral necrosis in the early hours following recovery, some animals may sustain blood pressure lowering to levels as low as 26-28 mm Hg without sequellae. This inconstancy in outcome suggests that some variable other than magnitude of oxygen deprivation defines brain pathologic response to hypoxia. These issues are further complicated by the fact that exposure to total oxygen lack (anoxia) does not cause late brain edema or injure structures in the hemispheres. Instead, tracheal occlusion or cardiac arrest for 14-24 minutes injures nuclear structures in brain stem (Myers, R.E.: Arch Neurol 29:394, 1976). These marked differences in response to anoxia versus hypoxia are puzzling especially since hypoxia may cause more devastating injury to brain than anoxia.

Recent studies have pin-pointed that critical variable which uniquely determines the brain pathologic response to anoxia or hypoxia. While arrest of circulation in food-deprived monkeys for as long as 12-14 minutes produces no brain injury, arrest of the same duration in normally fed or glucose-infused animals leads to catastrophic damage to the brain associated with blood-brain-barrier breakdown, cerebral edema, and widespread cerebral tissue necrosis appearing in the early hours after recovery (Myers, R.E.: Neurol 26:345, 1976). The principal difference in brain biochemical response to circulatory arrest produced by prior feeding or glucose infusion is lactate accumulation in excess of 15-20 umoles/gm (Myers, R.E. and Yamaguchi, M.: J Neuropath Exper Neurol 35:301, 1976). Thus, accumulation of a large excess of lactate within brain tissue seems to be the primary cause of the observed delayed breakdown in blood-brain-barrier function, development of brain edema, and widespread tissue necrosis. This interpretation is further supported by the fact that severe hypoxia which causes similar brain pathologic changes also leads to accumulation of lactate in brain at high concentrations (Yamaguchi, M. and Myers, R.E.: J. Neuropath Exper Neurol 35:302, 1976). It is concluded that dietary history with respect to prior carbohydrate intake critically determines brain pathologic response both to anoxia and hypoxia. The mechanisms leading to lactate accumulation during oxygen deprivation is reduced citric acid cycle activity causing pile-up of pyruvate as available glucose is degraded through the Emden-Meyerhof pathway. However, excessive tissue pyruvate is converted to lactate which, in the absence of oxygen, accumulates in stoichiometric relation to available carbohydrate stores. In anoxia which causes early arrest of circulation the quantity of lactate accumulated largely depends on preexisting brain tissue carbohydrate stores while in hypoxia, lactate is free to accumulate in large quantities even though initial tissue carbohydrate stores may be low because a preserved circulation continues to supply exogenous glucose for uninterrupted entry into the Emden-Meyerhof pathway.

1044 HYPERTHERMIA EVOKED BY ACUTE MECHANICAL DAMAGE TO THE HYPOTHALAMUS AND ITS ANTAGONISM BY INDOMETHACIN. Thomas A. Rudy, John W. Williams* and Tony L. Yaksh. School of Pharmacy, University of Wisconsin, Madison, WI 53706.

Both clinical experience and animal experimentation suggest that cerebral trauma, especially when the hypothalamus is involved, can evoke extreme hyperpyrexia. There is also evidence that traumatization of cerebral tissue can initiate the release of endogenous prostaglandins. Prostaglandins of the E series, when injected intracerebrally, cause intense hyperthermia by an action within the anterior hypothalamic/preoptic region. As part of a continuing series of investigations of the factors involved in the production of cerebral trauma-induced fever (CTF), we have developed a simple and reliable animal model of CTF and have examined the effect on these fevers of the prostaglandin synthesis inhibitor, indomethacin.

Male albino Holtzman rats weighing 250-400 g were each prepared with a single intracerebral guide cannula (18 ga stainless steel tubing, 9 mm long) implanted with its tip at stereotaxic coordinates (Pellegrino and Cushman atlas) AP 7.0, L 0.5, H + 2.0. The cannula was occluded at surgery with a solid 23 ga stainless steel stylet the same length as the guide. Two weeks were permitted for recovery from surgery. Each animal was then placed in a restraining device at an ambient temperature of 24°C, and colonic temperature (T_c) was monitored for at least one hour or until it had stabilized. The indwelling stylet was then removed and replaced with one 6 mm longer than the guide tube. The puncture trauma (PT) thus induced obliterated unilaterally the rostral portion of the hypothalamus and much of the preoptic region. The hyperthermia elicited by PT was quantified on the basis of both the maximum rise in T_c observed within 6 hours after PT (ΔT_c, in °C) and the Fever Index (FI), the area beneath the fever curve during the 6 hours following PT (in °C-hour). Latency (L, in minutes) to onset of hyperthermia was also measured.

Twelve rats which underwent PT with no pretreatment experienced an immediate and long-lasting hyperthermia ($L = 0.0 \pm 0.0$; $\Delta T_c = 2.34 \pm 0.13$; $FI = 10.19 \pm 0.79$). After 8 to 24 hours, T_c usually returned to the pre-PT level or to a slightly lower level. Twelve animals pretreated with 5 mg/kg indomethacin i.p. 1 hour prior to PT experienced a significantly attenuated hyperthermia ($L = 4.00 \pm 2.63$; $\Delta T_c = 1.44 \pm 0.25$; $FI = 5.15 \pm 1.10$). Pretreatment of 12 rats with 15 mg/kg indomethacin almost abolished the fever produced by PT ($L = 14.33 \pm 9.10$; $\Delta T_c = 0.46 \pm 0.10$; $FI = 1.20 \pm 0.39$). An additional group of 12 animals pretreated with the vehicle for indomethacin (40% normal saline/60% DMSO) exhibited a hyperthermia which did not differ significantly from that experienced by the PT-only group ($L = 2.64 \pm 0.80$; $\Delta T_c = 2.08 \pm 0.28$; $FI = 9.11 \pm 1.54$).

These findings indicate that the model of CTF described above elicits reliably a uniform hyperthermia which can be employed in further studies of the CTF phenomenon. The extensive and dose-related attenuation of the effect afforded by indomethacin pretreatment suggests strongly that the fevers were mediated ultimately by the release of endogenous prostaglandins. Thus, at least some forms of CTF may not be "neurogenic" in the traditional sense of the term. (Supported by NIH grant No. NS 11175-03 and Office of Naval Research Contract No. N00014-75-C-0939, NR 201-115.)

1045 LATENT HERPES SIMPLEX VIRUS (HSV) INFECTION OF MOUSE GANGLIA
J. Schwartz, W. O. Whetsell,Jr.* and T. S. Elizan. The Mount Sinai School of Medicine, Dept. Neurol., New York City, N.Y. 10029.

Suckling Swiss C-57 albino mice were inoculated in the right footpad with 10^4 infectious units of HSV (Mp strain, type 1). At various time intervals, the right and left sacral ganglia were placed into explant culture. HSV could be recovered from cultures of right (ipsilateral) ganglia for as long as 64 days after animal inoculation, even in the absence of clinical disease. Virus could not be recovered from cultures of left (contralateral) ganglia. The virus that was recovered was studied in Hela cells and in continuous tissue culture cell lines and showed characteristic cyto-pathic effects. To investigate if the recoverable virus had been altered in any way during latency, ganglia from infected mice were taken at 3 weeks, 2 months, 4 months, and 6 months following inoculation, homogenized, and placed onto differentiated organotypic cultures of mouse dorsal root ganglia (mDRG). In this system, light microscopic cyto-pathology was minimal. By electron microscopy, it was determined that only certain types of cells became infected: Nerve cells were uninvolved, while non-neuronal cells showed intranuclear virus particles; extensive cell fusion was not observed. The virus replication pattern appeared to be complete, but was limited to supporting Schwann cells. On direct HSV inoculation of similar mDRG cultures, this differential cell response was not observed.

These results suggest that in cultures of organized peripheral nervous system, non-neuronal cells initially allow persistence of HSV recovered from ganglia which were latently infected for up to 4 months.

Supported by NIH-NS-11631 and NINDS-75-04.

1046 FAST AXONAL TRANSPORT TO THE PACINIAN CORPUSCLE - A VULNERABLE STRUCTURE IN DISTAL AXONOPATHIES. Peter S. Spencer*, Marvin L. Sussman* Harold J. Weinberg* and Herbert H. Schaumburg*. (SPON: Cedric S. Raine). Depts. of Pathology and Neuroscience, Saul R. Korey Dept. of Neurology, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, The Bronx, New York 10461, and Dept. of Physiology and Biophysics, University of Miami School of Medicine, Florida.

The normal structure and function of mammalian nerve terminals is assumed to be dependent on a constant supply of materials arriving from neuronal perikarya. Any abnormality in the synthesis or transport of these materials might result in a decreased supply which would lead to a pathophysiologic alteration of the nerve terminal. Such an alteration has been demonstrated to occur in pacinian corpuscles of animals treated with neurotoxic compounds. For example, after systemic intoxication with acrylamide, there is a selective loss of the generator potential followed by a series of structural alterations which culminate in axonal degeneration. The present study was undertaken (a) to demonstrate transport of materials from neuronal perikarya to pacinian corpuscles in normal animals, and (b) to establish a model with which to examine the effects of neurotoxic chemicals on this process.

For this purpose, a total of ~10 µl of buffered ^{35}S-methionine (100-400 Ci/mmole) was injected via a glass micropipette into those lumbar spinal ganglia (left L2 and L3) which supply pacinian corpuscles in the mesocolon (Lee, F.C.: J. Comp. Neurol., 64:497, 1936) in each of 6 cats. Anesthetized animals were maintained at constant body temperature for periods of 5-7 h to allow label to arrive in the mesocolon. After perfusion with fixatives, up to 10 pacinian corpuscles, some with their attached afferent nerve fiber, were removed both from the mesocolon and, for control purposes, from the central toepads of the forefeet and the right hindfoot. Cross-section autoradiograms prepared from mesocolon corpuscles revealed, at all time-points, a large number of grains over the bulbous ultraterminal axon and sparse grains over the elliptical terminal. Autoradiograms of corpuscles obtained from the feet revealed no specific axonal labeling suggesting that background contamination was negligible.

In order to determine the pattern of movement of incorporated ^{35}S-methionine along sensory nerve fibers, an additional ~10 µl of label was introduced into the left L7 spinal ganglia of four cats. After perfusion, the distribution of label in 3 mm segments of the right and left sciatic nerves was studied by liquid scintillation spectrometry. The usual waveform of fast axonal transport, with a front moving at ~400 mm/day, was demonstrated in the left sciatic nerve and a low background in the right sciatic nerve.

This study provides evidence for delivery of labeled proteins to pacinian corpuscles by fast axonal transport and offers a model to investigate the systemic and local effects of substances on single, identified, afferent terminals.

Supported by grants from American Cyanamid Company, Dow Chemical Co., Vistron Corporation and Nalco Chemical Company, and by U.S.P.H.S. grants OH 00535, NS 03356 and NS 08952.

Dr. Spencer is the recipient of a Joseph P. Kennedy, Jr., Fellowship in the Neurosciences.

1047 HISTOLOGIC CHANGES DUE TO CHRONIC ELECTRICAL STIMULATION OF THE CAT CEREBRAL CORTEX. Suzanne S. Stensaas and Cameron J. Schlehuber. Dept. Anat., College of Med., Univ. of Utah, Salt Lake City, Utah 84132.

The effects of long term electrical stimulation of the cat cerebral cortex through an indwelling electrode array was studied. Histological changes at various time periods were correlated with biphasic and monophasic capacitively coupled waveforms.

Twenty-nine cats were implanted with a platinum electrode array containing seven 1mm discs embedded in Teflon. Three weeks after implantation daily stimulation was begun 12 hours per day. The stimuli consisted of 2.5 ma pulses at 50 Hz delivered through a 1μF capacitor; the pulse polarity was either (1) positive, (2) negative, or (3) paired positive-negative. Total stimulation time ranged from 24 to 492 hrs. Following stimulation, the cats were anaesthetized, perfused with a buffered aldehyde fixative and samples of tissue removed and embedded in Araldite. For each waveform tested, a total of forty two samples of tissue were prepared for histological analysis. Semithin (1μ) sections were cut from tissue lying near the center of each electrode. Tissue from each animal was also prepared from the contralateral unstimulated cortex, under the reference electrode, and under the Teflon paddle. An arbitrary scale of 1-5 was used to indicate histological changes in: meningeal connective tissue, subarachnoid space, subpial and cortical astrocytes, total thickness of layers I-V. The incidence of macrophages, dark hematogenous cells, dendritic swelling and axonal degeneration was also rated.

It was observed that the electrode array was always encapsulated by meningeal cells and connective tissue containing plasma cells, granulocytes and macrophages. The capsule was thicker under stimulated electrodes than under non-stimulated electrodes and a low correlation was noted between capsule thickness, occlusion of the subarachnoid space, and cortical damage.

Early (90-144 hrs) reactive changes observed by light microscopy included dendrite swelling, myelinated axon degeneration and infiltration of the cortical surface and perivascular space by small dark cells. Astrocyte hypertrophy and loss of normal neuropile texture in layer I were visible after long periods ofstimulation (300-500 hrs).

Ultrathin sections of stimulated cortex examined by electron microscopy displayed a loss of neuropile in layer I. Reactive astrocytes near the surface of the cortex contained increased glycogen, fibrils and residual bodies and were united by a greatly increased glycogen, fibrils and residual bodies and were united by a greatly increased number of zonulae occludens. Axonal processes with clear and dense core vesicles characteristic of axon sprouts were common in layer I of animals receiving more than 324 hours of stimulation (> 27 days). Normal axo-dendritic synapses were rare and the dendrites lacked microtubules, filaments and appeared smaller and denser than normal. Blood vessels appeared normal. It should be noted that these changes were seen with all 3 waveforms and were maximal under the reference electrode.

Slight differences in the effects related to the 3 waveforms were seen in cortical layers I-III. The changes did not appear to progress with time; in fact, fewer changes were apparent after 240-360 hours of stimulation than after shorter periods (24-180 hrs). This is interpreted to mean that the products of initial damage were removed and were followed by a stable condition. Similar stimuli (at a mean threshold amplitude of 1.67 ma) are being used for brief periods of stimulation of human visual cortex (Nature 259:111, 1976). We infer that no further damage can be expected from continued stimulation of these electrodes in humans at levels in the 1-2 ma range during continuing studies since each electrode to date has received approximately 15 mins of stimulation.

1048 OZONE AND VISUAL EVOKED POTENTIALS IN RATS. Bertram Berney*, Robert S. Dyer and Zoltan Annau (SPON: L.D. Fechter). Dept. Environmental Med., Johns Hopkins Univ., Baltimore, MD. 21205.

Recently it has been suggested that O_3 may affect processing of information by the visual system. The present experiment was designed to test this suggestion. Rats were chronically implanted with bipolar nichrome wire electrodes in the superior colliculus (SC) and screw electrodes in the skull for recording the cortical evoked potential and for grounding the animal. Following recovery (1 wk), recording sessions began. The rats' pupils were dilated with atropine, and the animals were placed in a recording chamber with mirrors on 3 walls and a strobe lamp on the fourth wall. Compressed air was blown into the chamber at 7 liters/min. A mean of the evoked responses to 500 light flashes presented at 0.4hz was obtained, and the compressed air was immediately supplemented with O_3 at either 0.00, 0.50, 0.75 or 1.00 ppm. The O_3 exposures continued for about 2 hr and 21 min, the last 21 min containing another series of 500 flashes. Latencies and amplitudes of different peaks obtained from the second series of flashes were taken as a percentage of the values obtained from the first series, and these percentages were compared across the different exposure levels. At least 1 wk elapsed between same-animal exposures. The results supported previous reports that the SC evoked potential is more reliable than the cortical evoked potential as a mirror of toxin-induced changes. At 1.00 ppm amplitudes and latencies of early SC peaks increased. The increased amplitudes may reflect a reticular induced arousal triggered by the irritating effect of O_3 on the nasal mucosa. The increased latencies are difficult to understand, but may also reflect the irritant properties of O_3.

1049 TEMPORAL ANALYSIS OF AXONAL PATHOLOGY FOLLOWING BLUNT CONTUSION OF THE SPINAL CORD OF THE RHESUS MONKEY (Macaca mulatta). AN ELECTRON MICROSCOPIC STUDY. Bresnahan, J.C. Department of Anatomy, The Ohio State University, Columbus, Ohio, 43210.

Following contusion at upper thoracic levels, the spinal cords of eight rhesus monkeys were examined with the electron microscope. Survival times ranged from 4 hours to three weeks. Samples were taken from the lesion site, from areas 3 and 10 mm rostral and caudal to the lesion center, and from the lumbosacral cord.

Four hours postoperatively, several small axons located close to the grey at the lesion site exhibit abnormal accumulations of organelles including mitochondria, dense bodies, vesicular structures, and multivesicular bodies. By 12 hours postoperatively many axons at the lesion site appear to be swollen with organelles and exhibit a thinning of their myelin sheath. Some organelle-rich profiles lack a myelin sheath altogether. At this time there are dark axons, and myelin sheaths which appear to be empty or to have small amounts of flocculent material in them. By 18 hours there are the first signs of axonal changes in the tissue taken 3 mm from the center of the lesion (both swollen and pyknotic axons are present). The axonal pathology spreads over time from the central part of the cord to the periphery at the impact site, and from the impact site rostrally and caudally beginning at 18 hrs. and continuing for the duration of the study. Small fibers degenerate first and large fibers degenerate later.

The axonal changes observed appear to be generally comparable to those reported for the peripheral nervous system in other species. The main difference is that we have no strong evidence to suggest that regeneration of axons at the lesion site occurs. (Supported by NIH grant, NS-10165.)

1050 METHODOLOGICAL PROBLEMS IN RESEARCH ON SUBCLINICAL LEAD POISONING IN CHILDREN. Samuel D. Brinkman* (SPON: D. L. Chute). Dept. of Psych., Univ. of Houston, Houston, Tx., 77004.

Current research on subclinical lead poisoning in children has been plagued by methodological problems. Pharmacological and biochemical studies indicate that researchers may not have adequately separated high lead groups from control groups. An alternate method, employing serial samplings of amino-levulinic acid dehydrase (ALAD), as well as lead in whole blood (Pb-B), is proposed. Based on animal and human research, a model of bilateral, diffuse subcortical demyelination and widespread biochemical upset is proposed as the expected effects of lead intake. The use of an established, highly standardized neuropsychological test battery is recommended to allow the subject to serve as his own control, and to reduce intersubject variability. A multivariate statistical procedure is proposed to facilitate comparison of overall central nervous system functioning in place of a series of comparisons of discrete behavior areas, as has been reported in the literature.

1051 MORPHOLOGICAL ABNORMALITIES IN CNS MYELIN FORMATION PRODUCED BY CYCLOHEXIMIDE. M. J. Cullen* and H. deF. Webster (SPON: M. R. Murray). NIH, Bethesda, MD. 20014.

Optic nerves of Xenopus tadpoles were exposed to cycloheximide in order to study the morphological effects of inhibiting protein synthesis on the formation of CNS myelin. Groups of stage 52-55 tadpoles received a 5 µl subcutaneous injection of 1.5×10^{-3} to 7.1×10^{-6} M cycloheximide or were immersed in a 7.1×10^{-5} M solution. After 12-72 hrs., the tadpoles were sacrificed and their optic nerves were prepared for electron microscopic study. After 12 hrs., there were severe alterations in oligodendrocytes. The number of polyribosomes in their perikarya and processes was greatly reduced and the granular endoplasmic reticulum was disorganized. Mitochondria, profiles of smooth endoplasmic reticulum and microtubules were normal in appearance. In addition, many oligodendrocyte tongue processes at the inner margins of myelin sheaths were enlarged, occasionally indented axons, and were filled with vesicular profiles. Focal changes in the lamellar structure of myelin were found in paranodal regions. The internodal portions of myelin sheaths, axons, and astrocytes appeared normal. These observations suggest that cycloheximide, by inhibiting protein synthesis, alters oligodendrocytes' ability to synthesize and insert membrane components into CNS myelin. This process is also temperature dependent since similar lesions have been produced in oligodendrocyte tongue processes by maintaining tadpoles at 4-10°C during myelin formation.

1052 ULTRASTRUCTURAL CHANGES FOLLOWING EXPERIMENTAL CEREBRAL ISCHEMIA IN THE GERBIL. Ronald F. Dodson, K.M.A. Welch and Lena W-F. Chu.* Departments of Neurol. and Pathol., Baylor Coll. Med., Houston, Tx. 77030.

Acute response of cerebral tissue was studied in the gerbil model in which groups of animals were subjected to right common carotid occlusion for respective periods of either 5, 10, 15 minutes; 1, 3 or 6 hours. Then the clip was removed from the artery and the animals were divided, where possible, into further subgroups according to presence or absence of neurological deficit. The animals were then subjected to whole body perfusion with 3% glutaraldehyde in 0.1M phosphate solution. Cortical and basal ganglia areas were sampled from both hemispheres, processed through standard osmication/dehydration procedures and embedded in Spurr plastic. Tissue response was noted to follow the sequence of events we have reported in primate studies (1,2). Edema was also observed in those non-neurologically deficient animals subjected to 3 or more hours of vascular occlusion. Involvement was limited to the perivascular region and consisted of intracellular swelling within astrocytic foot processes. Data indicated that neurological deficiency was related to an extension of morphological involvement beyond the perivascular astrocytic compartment.

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1053 MAST CELLS IN THE BRAIN OF MAN. John J. Dropp. Dept. Biol., Wilson College, Chambersburg, PA 17201.

Formalin-fixed, celloidin-embedded, serially-sectioned (35um), cresyl violet-stained sections of the brains of ninety-two human beings (45 "normative" brains, 26 brains with either surgically placed or focal cerebrovascular lesions and 21 brains from individuals with congenital or acquired encephalopathies) of the Yakovlev Collection of the Armed Forces Institute of Pathology are being examined for mast cells (MC's). So far, they have been found to be most numerous and most consistently present in the infundibulum, pineal, area postrema, and choroid plexuses. In the area postrema of males and in the infundibulum and choroid plexuses of both sexes their numbers decrease slightly with age. No such phenomenon is observed in either the area postrema of females or the pineal of both sexes. In addition, MC's are more abundant and more consistently present in the area postrema of males at all ages.

Except for the leptomeninges over the pineal and infundibulum, where MC's are numerous in several individuals, they are rare or absent in these membranes elsewhere in the brain. Occasional MC's are also seen within the supraoptic crest, subfornical organ and ventricles (I, II, III, and IV) in a small number of brains. Small numbers of MC's were also present in the cerebral cortex of one individual (88 yr. male). (Supported by NINCDS Grant-No 1-NS-4-2303.)

1054 VIRUS-INDUCED MODIFICATION OF CATECHOLAMINE METABOLISM IN THE DEVELOPING RAT BRAIN. Ras B. Guchhait* and Andrew A. Monjan. Dept. of Epidemiology, Sch. of Hygiene, JHU, Baltimore, MD. 21205.

The pathogenic manifestations of viral infections of the CNS have typically been examined at the morphological level. These studies were conducted to determine how an infection of the developing nervous system would affect the ontogenesis of neurochemical pathways. As a model system, we examined catecholamine metabolism by determining brain levels of monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) activity in rats infected intracerebrally with lymphocytic choriomeningitis virus (LCMV); an infection which results in an immune-mediated lesion of the cerebellum and for which the pathogenesis has been well characterized. LCMV produced no alterations of MAO activity. However, there was a significant increase of COMT activity (from 10η moles/mg/hr for normal to 20η moles/mg/hr for infected rats) which paralleled the time course of the active lesion process. Although most of this increase was within the hind brain, there was also a significant increment of COMT activity (57% above normal) in the forebrain, which carries virus but shows no necrotic lesion. The regional distribution of the enzymes in the normal adult rat brain is not uniform; COMT activity is greatest within cerebellum and brain stem while MAO activity is highest in diencephalon and cerebral cortex. Six months after LCMV infection, elevated levels of COMT activity was still evident within the residual cerebellar tissue (20.4η moles/gm/hr for infected vs. 11.7η moles/mg/hr for normal rats) while forebrain values were no longer different. Whether this virus-induced modulation of catecholamine metabolism reflects a response to the tissue damage or a more direct virus stimulation of these pathways remains to be elucidated.

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1055 TRANSPLACENTAL INDUCTION OF CHRONIC HYDROCEPHALUS IN FERRETS WITH METHYLAZOXYMETHANOL ACETATE (MAM Ac). R. Haddad, J. Shek, A. Rabe, S. Donahue, and R. Dumas*. Institute for Basic Research in Mental Retardation, New York, N. Y. 10314.

MAM Ac given the pregnant ferret (15 mg/kg IP) on day 32 of gestation (full term is 42 days) results in lissencephaly and hydrocephaly in all of the kits. At six weeks of age, failure of normal convolutional development of the cerebral cortex and dilatation of the lateral ventricles were seen in each of ten kits (from four litters) sacrificed at weaning. In most, a substantial dilatation of the fourth ventricle was also observed. Many cerebellar anomalies were found: abnormal persistence of the external granular layer, irregularities of the internal granular layer, nests of ectopic granule cells in the molecular layer and in the interlobular fissures, and a considerable number of displaced Purkinje cells. Kits sacrificed between 10 and 20 weeks of age were found to have more pronounced lissencephaly and hydrocephaly, but no neurological deficits were observed during this period. Fourteen treated ferrets were maintained to more than one year of age. All had severe cognitive impairment in comparison to age matched control animals, but only three had intermittant neurological signs. On some days they were unable to use their hind legs. Walking was accomplished with the fore legs alone, the hind legs being dragged limply behind. On sacrifice, all the treated animals were found to be severely hydrocephalic. The three with the most extreme distention of the cerebral hemispheres showed a flattening of the anterior aspect of the cerebellum All but the two least affected animals were found to have dilatation of the fourth, as well as the lateral, ventricle. Body weights were normal. There were no visceral anomalies.

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1056 INTRACEREBRAL HEMORRHAGE INDUCED BY PROSTAGLANDIN E_2 IN ESTROGEN PRIMED FEMALE RATS. Nicholas R. Hall and William G. Luttge. Dept. Neuroscience, University of Florida College of Medicine, Gainesville, FL. 32610.

Prostaglandins have been implicated in the mediation of a variety of vascular processes while estrogen can decrease the sensitivity of platelets to aggregating agents as well as regulate brain levels of certain prostaglandins. To investigate possible interactions between estrogen and prostaglandin E_2 (PGE_2) in the induction of intracerebral hemorrhage, a group of ovariectomized female rats was primed sc with 2 ug of estradiol benzoate. Crystalline PGE_2 (8 ug total) was implanted bilaterally into the anterior hypothalamus-preoptic area. Within 30 minutes, 60 percent of the animals were ataxic, were experiencing loss of the righting reflex and were extremely lethargic as measured in an open field activity chamber. Within 6 hours, all but one of the animals exhibiting these symptoms were dead. At autopsy, massive intracerebral hemorrhages were found in the vicinity of the implant site. Bilateral implantation of cholesterol filled cannulas had no behavioral or physiological effects in a group of control animals. Unilateral implants of 4 ug of PGE_2 also had no adverse effects when implanted in the anterior hypothalamus, medial basal hypothalamus or anterior mesencephalon. However, 2 out of 7 animals that received intraventricular PGE_2 were found to have small intracerebral hemorrhages following sc dexamethasone (250 ug/kg).

Supported by grant (HD-07049) to WGL and pre-doctoral fellowship (MH-05114) to NRH. PGE_2 was generously provided by the Upjohn Company, Kalamazoo, Michigan.

1057 ANTIBODIES TO DISSOCIATED CEREBELLAR CELLS IN NEW ZEALAND MICE AS DEMONSTRATED BY IMMUNOFLUORESCENCE. Steven A. Hoffman*, Andree A. Hoffman*, Ronald J. Harbeck*, and David Wm. Shucard. Dept. Medicine and Psychophysiology Research Laboratories, National Jewish Hospital and Research Center, Denver, Colorado 80206.

The occurrence of an antibody in New Zealand Black (NZB) mice to dissociated cerebellar cells from 6-10 day old BDF_1 mice was detected by indirect immunofluorescence. The sera from NZB mice displayed a significantly higher binding to cerebellar cells by this technique than did the sera from a group of mice from two non-autoimmune strains (BDF_1 and CAF_1). The NZB sera showed a positive correlation between an index of cytotoxicity to cerebellar cells and the percentage of serum immunoglobulin (Ig) binding to the cerebellar cells. The NZB sera also showed a significant positive correlation between IgM binding and cytotoxicity to cerebellar cells. No relationship was seen between IgG_1 and IgG_2 fluorescence and the index of cytotoxicity. There was no positive correlation between Ig binding to dissociated cerebellar cells and levels of thymocytotoxicity for any of the tested classes of immunoglobulin in any of the mouse strains used.

Sephadex G-200 fractionation of NZB sera positive for Ig binding to cerebellar cells showed this activity in peaks I (void volume) and II of the three major peaks. Pooled normal mouse sera showed elevated Ig fluorescence in only the second peak. In all cases, the heightened fluorescence was determined to be IgM when the appropriate fluoresceinated antisera was used.

The data suggest the presence, in some NZB mice, of an IgM antibody reactive with components of the central nervous system.

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1058 CEREBRAL BLOOD FLOW IN MONKEY FOLLOWING SPINAL CORD INJURY. W.E. Hunt, W.G. Bingham, L. Sirinek*, K. Crutcher, C. Mohnacky*. Div. Neurosurgery, Ohio State Univ. Med. Sch., Columbus, Ohio 43210

Blunt trauma to T6 segment of monkey spinal cord sufficient to produce severe persistent paraplegia and central hemorrhagic necrosis resulted in alteration of blood flow throughout entire spinal cord, but had no demonstrable effect on cerebral blood flow. Using C14-antipyrine indicator fractionation technique, blood flow was determined for 31 areas of brain and gray and white matter of several segments of spinal cord and expressed as a function of time from 5 min to 12 hrs post trauma. Flow in central gray of traumatized cord segment was obliterated while flow in white matter fell to 50% of control in 30 min and rose to 60% over the ensuing 12 hrs. Flow in remainder of cord fell to approximately 60% in cervical area, T2 control segment and lumbar area in 30 min and returned to 90% in 12 hrs. Cardiac output also fell to 70% in the first hr, returning to 90% in 12 hrs. Flow throughout brain was unaffected by spinal cord injury and flow values compare favorably with those published by other investigators on anesthetized animals using antipyrine and hydrogen electrode techniques; e.g. motor cortex remained at 65 ml/100g/min; putamen 62; thalamus 53; central white matter 22; medullary reticular formation 48. Autoregulation of spinal cord blood flow appeared to be affected by localized mid thoracic trauma while that of brain remains intact.

1059 CHANGES IN CEREBROSPINAL FLUID PRESSURE ASSOCIATED WITH FEVER. Jackson-Middelkoop, L.M.*, Veale, W.L., Cooper, K.E. and LeBlanc, F.E.* Spon: R.G. Lee., Divisions of Medical Physiology and Surgery, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

Fever of rapid onset is frequently associated with headache and hyperthermia is often found in patients following head injuries. We have undertaken a series of experiments in order to examine the relationship between cerebrospinal fluid (CSF) pressure and experimentally-induced fever. Cats weighing between 3-3.5 kg were prepared aseptically with guide tubes bilaterally, the tips of which rested directly above the lateral cerebral ventricle. In addition, a catheter was inserted into the jugular vein in order to permit the intravenous application of pyrogen. To record cerebrospinal fluid pressure, a needle was passed through one of the guide tubes so that the open end rested within a lateral cerebral ventricle. Body temperature was recorded continuously by means of a thermistor probe placed in the colon. Fever was produced by the injection of 0.05 mg SAE pyrogen injected directly into the blood stream. All experiments were carried out on fully awake, unrestrained cats.

At the onset of the fever the animal vasoconstricts and takes a behavioral posture consistent with the maintenance of heat. On occasion shivering can be observed. During this time CSF pressure increased sharply and remains elevated until the fever "breaks". At the time body temperature begins to return to normal, the animal moves from a curled position, often eats, vasodilates, and shows an increase in respiratory rate. At this time CSF pressure decreases to the pre-injection level. The mechanisms by which fever may alter CSF pressure and the clinical implications of these observations will be discussed.

This work was supported by the M.R.C. of Canada. L.J.-M. is supported by the Medical Trust Fund of the University of Calgary.

1060 EXOGENOUS FACTORS AND ALZHEIMER'S DISEASE. L. Liss, D. Couri*, K. Ebner*, E. Young*, L. Socarras*. College of Med., Dept. Path. and Pharmacol., Ohio State University, Columbus, Ohio 43210 and Chillicothe VA Hosp., Chillicothe, Ohio 45601.

In the Alzheimer's Disease (AD) the neurofibrillary tangles occur simultaneously with elevation of aluminum levels in the areas of the brain affected by the disease. Brain regions in which there are no neurofibrillary tangles have normal levels of aluminum. Our past studies have shown increase in aluminum and formation of tangles in areas with altered Blood Brain Barrier (BBB) resulting from chronic pressure. Subsequently the animal model for neurofibrillary tangles was developed utilizing the incompetent BBB of the newborn. In the present study human autopsy material was evaluated for the modification of morphological picture of AD by conditions resulting in alteration of the BBB. Our results indicate that (a) the preexisting AD can involve areas which have been damaged by another process and are not involved in uncomplicated cases, or (b) two distinct types of morphological changes were found: The typical changes involving the regions usually affected in AD and atypical changes characterized by neuronal breakdown and massive sudanophilia in regions not commonly involved in AD. The most impressive was the coexistence of typical plaques and atypical plaques characterized by sudanophilia and disintegrating neuronal components observed in a patient with AD who survived a CO poisoning for 3 years. Our findings based on correlation of morphological changes and levels of aluminum in patients with strokes, Wernicke's disease, post-traumatic and following X-radiation indicate that an incompetent, altered or damaged BBB permits the penetration and elevation of the concentration of aluminum. The hypothesis which based on previously reported experimental studies is applied now to explain the development of changes in human brains.

1061 EXPERIMENTALLY INDUCED ISCHAEMIC CHANGES IN THE RAT OLFACTORY BULB. A GOLGI STUDY. Jesus Machado-Salas*, Madge Scheibel* and Arnold Scheibel. Dept. Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

In attempting to determine the appearance of early neuronal and/or neuropilic changes due to ischaemia, unilateral ligation of the common carotid artery was performed, under ether anaesthesia, in 20 rats.

The ischaemic periods lasted from 1.5 to 30 minutes. Afterwards the animals were decapitated and modification of the golgi methods were used to study this material.

Our observations in the olfactory bulb unilateral to the ligature (oclussion) have shown early distortion of mitral cell bodies (10 minutes) and swollen initial dendritic stalks, mainly of that stem oriented towards the olfactory glomeruli. Later, periodic lumpiness and constrictions, and dendritic fragmentations appear in mitral cells and internal granules.

Interestingly, the number of dendritic spines on the internal granules seems to be greater in the longer ischaemic cases than in the short ones. Quantitative evaluation is under way.

The value of golgi technique and of this model will be considered in the light of the development and evolution of ischaemic processes in the nervous system.

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Dr. A. Scheibel is supported by NIH grant NS 10567.

1062 ELECTRON MICROSCOPY OF SPINAL CORD INJURY: MODIFICATION OF REACTIVE EVENTS WITH IMMUNOSUPPRESIVES, PYROGENS, AND ANTIINFLAMMATORY AGENTS. M. A. Matthews, J. B. Gelderd and M. St. Onge. Dept. Anat., LSU Med. Ctr. and Nat'l Spinal Cord Injury Ctr., New Orleans, La. 70119.

Long-Evans hooded rats were subjected to a mid-thoracic transection of the spinal cord, then divided into 5 groups and treated with one of the following procedures: a) a single injection of Cytoxan; b) topical application of Piromen onto the transected cord with subsequent daily injections of this agent; c) daily injections of ACTH; d) a single, topical application of isobutyl-2-cyanoacrylate; e) no treatment. Animals were sacrificed at 7-180 d.p.o. followed by electron microscopic analysis of reactive events at the site of injury. This area is initially characterized by disruption of both the neuropil and surrounding white matter, together with influx and proliferation of leucocytes, fibroblasts and adventitial elements. These either incorporate and remove the products of neuronal degeneration, or participate in the production of a dense connective tissue septum, consisting primarily of coarse, irregularly oriented collagen bundles, within the defect left by the lesion. With longer survival periods, cysts form and enlarge, thus impinging into the rostral and caudal cord segments adjacent to the septum. Examination of those animals given Piromen or Cytoxan reveals alterations both in the septum, characterized by an areolar matrix of isolated, slender collagen fibers, and in the cysts, which are multiloculated by numerous vascularized septae. Additionally, fascicles of small, thinly-myelinated axons occur in abundance in these septae and within the loose matrix of the connective tissue septum. Present investigations are directed toward determining the cell bodies of origin and location of termination of these fibers. Supported by the Edward G. Schlieder Foundation and NIH Grant 25P30268.

1063 MODIFICATION OF BEHAVIOR ASSOCIATED WITH PERSISTENT VIRUS INFECTION OF THE RODENT BRAIN. Andrew A. Monjan and Steven D. Mark*. Dept. of Epidemiology, Sch. of Hygiene, JHU, Baltimore, MD. 21205.

Virus infection of the CNS may result in tissue destruction and consequent clearance of virus, or may result in little or no pathology and persistent infection. Neonatal infection of the rat with lymphocytic choriomeningitis virus (LCMV) results in a slowly developing lesion of the dentate gyrus of the hippocampus, the behavioral sequellae of which have been previously described. By the use of an immunosuppressive regimen of antilymphoid serum, the pathological effects of LCMV can be ablated with the establishment of the persistence of high levels of viral antigen throughout the neural parenchyma. Comparisons of the behaviors of virus carriers (VC), infected rats who have cleared virus but have a residual lesion (VL), and of normal controls (C) suggest that chronically infected neuronal cells may have functional alterations in the absence of gross pathology. In a 2-way shuttle-box avoidance task, both VC and VL rats avoid shock equally well as C, but have a different distribution of avoidance responses than C. VC and C perform similarly on DRL schedules, but the interresponse time histograms for VL indicate that these animals fail to inhibit short latency responses. VL and C rats exhibit the same diurnal pattern of spontaneous activity, while VC rats are less active and lose their diurnal pattern. Thus, these data indicate that persistent viral infection of neuronal cells can induce functional alterations, in the absence of apparent morphological changes, which may mimic the behavioral sequellae to specific lesions of the corresponding brain areas.

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1064 EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN MICE. Ilene N. Montgomery* and Helene C. Rauch. Dept. Immunol. and Microbiol., Wayne State U. Sch. Med., Detroit, MI 48201.

A study to determine the suitability of mice to investigate genetically associated immunologic parameters of EAE was undertaken. Mice are clearly advantageous in comparison to others species inasmuch as a large number of genetically characterized strains are available. Until the published report of Levine and Sowinski (J. Immunol. 110:139, 1973), mice appeared to be resistant to the induction of EAE. We have now induced EAE in several strains of mice following intradermal injection of mouse spinal cord tissue or myelin basic protein emulsified in Freund's complete adjuvant (containing heat killed virulent human tubercle bacilli) and immediate intravenous infusion of pertussis vaccine. We have explored the susceptibility of various strains using encephalitogenic preparations with and without pertussis vaccine and have evaluated the effect of age and sex on disease incidence. Adoptive transfer of disease using lymphocyte-rich cell suspensions has proved feasible. Other adjuvant preparations such as poly A:U, lipopolysaccharide have been compared with Freund's for their efficacy in inducing disease. Crosses were made between highly susceptible and apparently resistant strains and the susceptibility of the F_1 and F_2 offspring determined. No close association between the H-2 locus and susceptibility has been observed; neither does susceptibility to EAE appear to be correlated genetically with susceptibility to other autoimmune responses.

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1065 A HIGH INCIDENCE OF SPINAL TUMORS IN RATS FOLLOWING A SINGLE DOSE OF ETHYLNITROSOUREA. Michael J. Pfaffenroth* (SPON. Y. Nakajima). Dept. Biol. Sci., Purdue Univ., West Lafayette, IN 47907

Ethylnitrosourea (ENU) has been shown by a number of investigators to induce neurogenic tumors in 90-100% of rats that have received a single dose of ENU either transplacentally during the last week of gestation or during the first week of postnatal life. The incidence of such tumors within a single, restricted region of the central nervous system has, however, been much lower even though several sites of predilection have been reported. In studies on the teratogenic effects of ENU we noticed a predilection for spinal tumors in long-survival rats that either received a single dose of ENU during the last week of gestational development or that received a single dose of ENU administered directly to the cerebellum on day 6 of postnatal life.

In further studies 60 mg ENU/kg body weight were given iv. to pregnant laboratory-bred Wistar albino rats on day 20 of pregnancy and the offspring were examined for spinal tumors. Of 18 offspring born to these animals 15 developed a total of 20 spinal tumors in 4-8 months. A second group of animals had ENU injected directly into the cerebellum on postnatal day 6, and a third group had the same dose of ENU injected directly into the subarachnoid space of the cisterna magna. In each of these groups half of the animals developed spinal tumors in 6-13 months. In most cases these predominantly oligodendroglial tumors caused paralysis of the limbs for at least a week prior to death. The results of these studies agree with earlier reports concerning ENU carcinogenesis; however, the high incidence of spinal tumors in this stock of rats may make this model system a valuable addition to those already developed for the study of neurogenic tumors induced by alkylnitrosamides. (Research conducted under G.D. Das; supported by NIH Research Grant CA-14650.)

1066 THE PRODUCTION OF ANTISERA TO NEURONAL AND OLIGODENDROGLIAL SURFACE COMPONENTS. S.E. Poduslo, H.F. McFarland† G.M.McKhann
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Antisera produced to surface components of bulk isolated cells from brain can be used as markers for cell identification and for studies of proteins present in cell membranes. In addition bulk isolated cells can be used for absorption of known antisera to determine the specificity of the sera. Neurons, astrocytes, and oligodendroglia can be isolated from brain using established methods. With suitable washing, neurons and oligodendroglia can be maintained in tissue culture for several days. For antisera production, rat neurons were injected intravenously into rabbits while lamb oligodendroglia were injected subcutaneously; the injections were repeated at two week intervals. The specificity of the antisera was determined by immunofluorescence on maintained cells; both antisera react to cell surface components. Neither antisera can be absorbed with non brain tissue. The antiserum to neurons is absorbed with bulk isolated neurons, but not with rat myelin or equivalent weights of astrocytes. Mouse neurons also react. The antiserum to oligodendroglia is absorbed with oligodendroglia, but not with lamb gray matter or myelin. Calf and human oligodendroglia also cross react. Attempts to identify the antigens responsible are in progress. (Supported by funds from John A. Hartford Foundation; USPHS Grants # 10920, 08719; Multiple Sclerosis Society.)

1067 ABSENCE OF BRAIN EDEMA AFTER REVERSIBLE OSMOTIC OPENING OF THE BLOOD-BRAIN BARRIER. S. I. Rapoport, K. Matthews*, H. K. Thompson*, and K.D. Pettigrew*. NIMH, Bethesda, MD 20014.

Osmotic opening of the blood-brain barrier provides a means to test whether brain edema necessarily results from barrier opening and entry of plasma protein into interstitial brain space. Rhesus monkeys were perfused via the left lingual artery with 2.2 molal hypertonic solutions of DL-lactamide (Sigma Chemical Co.) which had been recrystallized from ethanol. Barrier opening on the homolateral cerebral hemisphere was determined by measuring extravasation of intravascular Evans blue-albumin, and graded from 0 to 3+. Only Grade 3+ opening was associated with neurological sequelae. Na, K and water contents of gray and white matters of perfused and unperfused regions of brain were determined 48 hr after perfusion, since we found previously (unpublished observations) that edema and ionic changes caused by higher concentrations of lactamide and urea, as well as by unrecrystallized lactamide, are maximal at this time. The barrier was opened to the Grade 2+ level, without neurological sequelae, in 7 of 8 animals, and no significant changes were measured in brain water and electrolyte composition in gray and white matters of perfused regions. Measurable edema may not have appeared because the barrier had closed without vascular damage by the time the measurements were made, the renewed impermeability to ions and low capillary hydraulic conductivity preventing significant capillary filtration. Furthermore, sufficient protein may not have entered the brain to increase tissue osmotic pressure and capillary filtration significantly, because of the nonlinear relation between osmotic pressure and concentration, or the protein which entered may have been metabolized or excreted from the brain parenchyma at 48 hr. Another possibility is that a low brain compliance prevented brain swelling in response to a small increase in capillary filtration rate.

1068 HEXACHLOROPHENE (HCP) INDUCED CNS MYELIN LESIONS STUDIED WITH FREEZE-FRACTURE AND ELECTRON-DENSE TRACER TECHNIQUES. Paul J. Reier, Takeshi Tabira* and Henry deF. Webster. Dept. Anatomy, Sch. Med., University of Maryland, Baltimore, MD. 21201 and NINCDS, NIH, Bethesda, MD. 20014.

Recent freeze-fracture studies of myelin sheaths have demonstrated the presence of numerous intramembranous particles. Random particle distribution and specialized arrangements associated with tight junctions were examined during the pathogenesis of intramyelinic, edematous lesions produced by HCP. Stage 54-58 Xenopus tadpole optic nerves were studied since: (1) their myelin sheaths develop lesions very rapidly, and (2) subcutaneously-injected tracers can penetrate the nerve parenchyma, thus providing an opportunity to analyze potential changes in sheath permeability in perspective with freeze-fracture observations. Replicas of nerves from tadpoles immersed in 0.2 µg/ml HCP solution for 1-10 days revealed that even chronic HCP exposure did not alter particle strands associated with tight junctions; these were easily recognized on the inner leaflet of membranes in extensively vacuolated sheaths. Following a 10 µl perineural injection of horseradish peroxidase (50mg/ml), the tracer was distributed throughout the parenchyma of the nerve; however, none was seen within myelin blisters. In contrast, some modifications occurred in the distribution of other intramembranous globules. Particle-free elevations, corresponding to multilaminar blebs projecting from myelin in thin sections, frequently occurred on leaflets adjacent to vacuoles; such were not seen in normal myelin of either control or HCP-exposed nerves. Thus, in spite of massive blistering caused by myelin splitting at the less dense, intraperiod lines, the integrity of tight junctions is preserved, and the impermeability of myelin to macromolecules is maintained. On the other hand, the presence of particle-free areas suggests that HCP may, to some extent, modify membrane structure. This observation, however, requires further investigation.

1069 HISTOPATHOLOGIC EVALUATION OF PARYLENE-COATED CHRONICALLY IMPLANTED MICROELECTRODES. M. Salcman, W. Whetsell* and M. J. Bak . Columbia University, Mt. Sinai School of Medicine, and NIH.

Long-term electrophysiologic recordings and the development of neural prostheses both depend upon the utilization of chronic microelectrode techniques in which the various biomaterials employed must demonstrate a high degree of tissue compatibility. A new electrical insulator, dichloro-di-para-xylene (Parylene), has been used to coat chronic recording intracortical microelectrodes made of pure iridium and gold (Salcman and Bak, Med. & Biol. Eng. 14:42-50, 1976). These have been implanted in the visual cortex of the cat and the animals harvested after intervals upwards of twelve months. Routine staining techniques (H & E, Masson trichrome, PTAH) reveal remarkably little gliosis and scarring in the brains of parylene implanted animals even after one year. Capsules are typically less than 15 µ in thickness and normal appearing neurons are regularly seen within 50 µ of the electrode tract. A relatively benign material, glass, incites an comparable tissue reaction at 3 months to that seen with parylene at twelve months. In consideration of other stringent mechanical and electrical requirements, parylene has proved itself to be a superior biomaterial for use in the central nervous system.

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1070 RETINAL TAURINE DEFICIENCY AND ERG AMPLITUDES. Susan Y. Schmidt*, Eliot L. Berson* and R. Bruce Szamier. Dept. Ophthalmology, Harvard Medical School, Boston, MA. 02115

Taurine, the predominant free amino acid in the retina, has been shown to be concentrated in the outer nuclear layer and by autoradiography appears to be within photoreceptors and Müller cells. Cats fed a casein diet devoid of taurine develop retinal taurine deficiency followed by photoreceptor cell degeneration. Decreases in the amplitudes of the a-wave and b-wave of the dark-adapted electroretinogram (ERG) and cone responses to 40 cps flickering light are linearly related to the decrease in the concentration of retinal taurine when retinal taurine is within 45-100% of normal. ERG responses become minimal or nondetectable when retinal taurine is reduced below 45% of normal. Ultrastructural abnormalities in the photoreceptor cells occur when retinal taurine concentration is reduced to 55% of normal. Photoreceptor cell death as evidenced by pyknotic nuclei in the outer nuclear layer and significant reductions in retinal DNA content occurs when retinal taurine concentration is reduced to 25-45% of normal. Supplementation of the casein diet with taurine prevents retinal taurine depletion and maintains normal ERG amplitudes, whereas addition of taurine precursors to the diet does not prevent the development of retinal taurine deficiency, ERG changes, and photoreceptor cell degeneration. These observations demonstrate that exogenous taurine is required by the cat and that widespread photoreceptor and/or Müller cell malfunction and subsequent photoreceptor cell death occur with retinal taurine deficiency.

1071 CATECHOLAMINE INVOLVEMENT IN THE PROCESS OF PROGRESSIVE HEMORRHAGIC NECROSIS OF THE SPINAL CORD. Ture W. Schoultz. Dept. Anat., Univ. Ark. Col. Med., Little Rock, AR. 72201

Norepinephrine (NE) has been implicated as being responsible for hemorrhagic necrosis of the spinal cord following trauma. To test this hypothesis 13 cats were catecholamine (CA) depleted (adrenalectomy plus reserpine). Nine were subjected to spinal cord trauma (400 gm-cm) and allowed to survive one hour; four were non-traumatized controls. Cords were analyzed for pathology and compared with 10 traumatized cords taken from cats which had not been depleted of peripheral CA. Six CA intact control cords were obtained. NE levels were measured in cord adjacent to the block removed for histology. Gray matter edema and perivascular spaces were of greater magnitude in CA depleted control cords than in CA intact controls, but the CA intact cords developed greater pathology of these types after injury than the CA depleted cords. Perineuronal spaces increased in CA intact cords after trauma; no change was observed in the CA depleted cord group. Periaxonal spaces increased in both groups. No change was observed in white matter edema. Presence of debris (white, gray matter), presence of PMN's (in vessel wall, in pia-arachnoid) and cellular changes (swelling, shrinkage, microvacuoles) were compared in traumatized cords of CA intact and CA depleted groups. Almost identical changes were observed after one hour; control groups were also identical. The CA depleted group demonstrated slightly greater vascular pathology (hematoma, diapedesis, petechial and flame hemorrhages). These data will be discussed as they relate to elucidation of the basic mechanisms underlying local destructive changes seen during the first hour after trauma.

1072 DEPRESSED CEREBRAL AUTOREGULATION IN PATIENTS WITH BRAIN ISCHEMIA. W. Schuler*, W. Hayward*, and E. Lichter* (SPON: I. Klatzo). Section of Neurological Surgery, Loma Linda University, Loma Linda, California.

The 25 patients undergoing microanastomosis for brain ischemia, cerebral autoregulation was measured. All patients had angiographically demonstratable lesions of the internal carotid or middle cerebral arteries, considered inoperable by the usual endarterectomy. Regional cerebral blood flow (rCBF) in gray matter was measured by the I.V. Xe^{133} technique previously described by Austin, et al. Arterial PO_2 PCO_2, and respirations were maintained constant. Following baseline measurement of rCBF, the mean blood pressure was increased an average of 30 mm Hg over a period of 15 minutes by an I.V. infusion of Neosynephrine. Following this, the rCBF was repeated. Normal variation of rCBF by the I.V. Xe^{133} technique is $\pm$ 14%. In this group of patients, the average increase of rCBF on the side of the lesion was 16 ml/100g/m or an average of 30%, significant of the $p < .001$ level. These results are interpreted as being consistent with a significant depression of cerebral auto regulation in the cerebral gray matter on the side of the lesion of patients with demonstrable ischemia. They further demonstrated the importance of maintaining an adequate blood pressure in order to prevent a subthreshold fall in rCBF and brain PO_2.

1073 CHRONIC IMMUNE COMPLEX DISEASE IN THE RAT: BEHAVIORAL AND IMMUNOLOGICAL CORRELATES. David Wm. Shucard, Steven A. Hoffman*, Ronald J. Harbeck*, Andree A. Hoffman*, Cheryl Beauford*, and Ronald I. Carr*. (SPON: H. Alpern). Dept. Medicine and Psychophysiology Research Laboratories, National Jewish Hospital and Research Center, Denver, Colorado 80206.

There is increasing evidence to suggest that immune complexes may play a significant role in the central nervous system (CNS) manifestations of systemic lupus erythematosus (SLE). In this investigation, an attempt was made to develop a model of immune complex disease in the rat in order to investigate the effects of this disease on central nervous system functioning and on subsequent behavior. Chronic immune complex disease was induced in rats by repeated injections of a sufficient quantity of bovine serum albumin (BSA) to keep the animals in antigen excess. Measures of proteinuria, the presence of immune complexes in the kidney and choroid plexus, and acquisition and extinction of avoidance behavior were obtained over a period of approximately 3 months. Differences in acquisition and extinction of avoidance behavior were noted between experimental and control animals. These behavioral effects were more pronounced in those animals showing the most elevated proteinuria. The presence of BSA appeared in immunofluorescence studies as patchy, granular deposits in the choroid plexus of experimental, but not control animals. The results of this preliminary investigation indicate that the rat may provide a useful animal model for the study of CNS involvement in immune complex disease.

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1074 VIBROTACTILE SENSITIVITY OF TRIGEMINAL NEURALGIA PATIENTS. Ronald T. Verrillo. Inst. for Sensory Res., Syracuse Univ., Syr., NY 13210, and Arthur D. Ecker. Community General Hospital, Syracuse, NY 13215.

Several hypotheses have been proposed which involve large myelinated sensory fibers in the production of pain in general and particularly in the pathogenesis of classical trigeminal neuralgia (tic douloureux). The purpose of the present experiments was to examine sensory responses to stimuli which normally activate large fibers. Vibrotactile thresholds of detectability at frequencies between 25 and 300 Hz were determined at the site of initial pain in 11 patients who had been or were currently being treated for neuralgia. In each case this site was the cheek bordering the nasolabial fold. Results: 1) Essentially no difference between tic and normal sides of the face prior to surgery. 2) The drug carbamazepine (Tegretol), commonly used to control pain of tic douloureux, did not affect vibrotactile thresholds. 3) Removal of the infra-orbital branch of the trigeminal nerve resulted in an initial loss of sensitivity at all frequencies followed by a gradual return of sensation, reaching preoperative levels in approximately one year. 4) Following alcohol injection into the juxtaganglionic trigeminal sensory root, low-frequency thresholds were elevated considerably and sensation above 100 Hz was lost completely. A recurrence of pain after years of relief following injection was accompanied both by the return of sensitivity at high frequencies and sensory improvement at low frequencies. Clinical examinations indicate that the return of sensation usually precedes the recurrence of neuralgia.

1075 PYRITHIAMINE-INDUCED ACUTE THIAMINE DEFICIENT ENCEPHALOPATHY IN THE MICE. Itaru Watanabe. VA Hospital, Kansas City, Mo. 64128 and Kansas University, Kansas City, Ks. 66103.

While it is very difficult to produce encephalopathy in the mouse by feeding a thiamine deficient diet alone, pyrithiamine, a thiamine antagonist can in conjunction with feeding of a thiamine deficient diet readily produce acute fatal brain distress similar to human Wernicke's syndrome. It has been known that pyrithiamine accumulates in the brain and inhibits transketolation and decarboxylation in the glucose and energy metabolism. However, a correlation between the biochemical and morphological pathogenesis still remains unexplained. Mature, 35 gm, male Swiss mice were fed ad libitum with a thiamine deficient diet (400 µg/Kg BW) on 4, 6 and 8. On day 10, the mice developed tonic seizures followed by ataxia and other vestibular signs. The brain showed multiple lesions of hemorrhagic necrosis in the vestibular nucleus, thalamus, mammillary body and mesencephalon. The morphological changes included extensive edema and necrosis of neuronal and glial cells. In the hemorrhagic blood vessels, mitochondrial swelling was prominent in the endothelial cells. By reducing the dose to 200 µg/Kg BW at the third injection, no neurological disease developed in the mice. No significant changes were seen in the brain of these mice. Thiamine treatment from day 9 prevented the illness, but was ineffective when started after the onset of encephalopathy. This irreversibility may relate with the pyrithiamine-induced severe damage of the brain tissue occurred simultaneously around the time of the clinical onset of the illness.

1076 EFFECTS OF ANESTHETICS ON HINDLIMB REFLEXES IN RATS PARALYZED WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS. Susan R. White. Fac.Med., Memorial University Newfoundland, St. John's, Newfoundland. A1C 5S7.

Complete hindlimb paralysis often accompanies the development of experimental allergic encephalomyelitis (EAE) in Lewis rats. White and Barnes (Brain Research, 84:123, 1975) found that lumbar spinal reflexes were obtainable in decerebrate EAE paralyzed rats while Baum and Rosenthale (Circ. Res. 18:118, 1966) could not obtain reflex contraction of tibial muscles in EAE paralyzed rats anesthetized with a barbiturate or with chloralose-urethane. Therefore, monosynaptic and polysynaptic reflexes measured from lumbar roots (L4 & L5) which contribute to the sciatic nerve in rats were examined in hindlimb paralyzed EAE rats anesthetized by drugs (chloralose, chloralose-urethane, sodium pentobarbital) or by precollicular decerebration. Electrical stimulation was applied to the central ends of cut L4 and L5 dorsal roots and hindlimb twitches were observed. Then L4 and L5 ventral roots were cut just before exiting the dura and placed across recording electrodes. Although the hindlimbs of the EAE rats were completely paralyzed, dorsal root stimulation produced leg twitches and monosynaptic and polysynaptic reflexes both in rats anesthetized with drugs and in precollicular decerebrate rats. Stimulus strengths required for elicitation of the reflexes were similar in EAE paralyzed and control animals. These results indicate that many spinal cord synapses involved in hindlimb reflexes remain functional in EAE rats with complete hindlimb paralysis.

1077 REVERSIBLE CEREBRAL ISCHEMIA IN GERBILS: CLINICAL, MORPHOLOGICAL AND BIOCHEMICAL CORRELATION. T. Yanagihara. Dept. Neurol. Mayo Clinic and Mayo Foundation, Rochester, MN. 55901.

In order to compare the progressive cerebral ischemia following permanent occlusion of a common carotid artery in gerbils (T. Yanagihara: STROKE 6:234, 1975), the same artery was closed for 30 minutes, and the recovery course of the symptomatic animals was studied. As observed in the case of permanent occlusion, the course was variable. The majority of the animals clinically recovered within 24 hours. Histological specimen from these animals revealed only minor pathological changes in cerebral cortex and thalamus, but various degrees of neuronal degeneration was observed in the pyramidal cell layers of hippocampus at CA3, CA2 and CA1 regions. The time course suggests that these changes were slow but progressive destruction of neurons. If the animals were not completely recovered, scattered ischemic changes were found in cerebral cortex and thalamus. The ability for protein synthesis was investigated 30 minutes following occlusion of the artery and during the recovery stage. Cerebral hemisphere and thalamus from the occluded side and the control side were obtained and protein synthesis was carried out with tissue slices in vitro with [^{3}H] lencine as a precursor. The nuclear, microsomal and soluble fractions were separated subsequently. After occlusion for 30 minutes, protein synthesis was decreased to less than 50% of the control values. The tendency for recovery was already observed 30 to 60 minutes following the re-establishment of circulation and at 18 to 24 hours, the value of the ischemic side was the same as the control side if the animal was clinically normal. The present investigation indicated close correlation of clinical, histological and biochemical changes during the recovery stage of cerebral ischemia, as was the case in the progressive cerebral ischemia. (Supported by Grant NS-06663 from NIH)

Neurotransmitters

1078 PENTOBARBITAL MODULATES AMINO ACID RESPONSES OF TISSUE-CULTURED MAMMALIAN SPINAL AND CEREBELLAR NEURONS. J. L. Barker and B. R. Ransom., Behavioral Biology Branch, NICHD, NIH, Bethesda, MD 20014.

The pharmacological effects of pentobarbital (PB) on neuronal membrane properties and amino acid responses at the single cell level have been studied using mouse spinal and cerebellar neurons grown in tissue culture. Conventional methods were utilized for intracellular recording and for iontophoresis of glutamate (Glu), γ-amino-butyric acid (GABA) and glycine (Gly). PB was either iontophoresed or allowed to diffuse from 0.1-0.2 mM solutions contained in blunt micropipettes. Mg^{++} was added to the bathing medium to eliminate synaptic activity and allow better evaluation of the pharmacology.

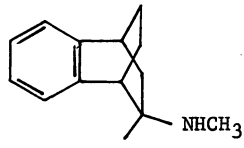
Glu invariably caused a dose-dependent depolarization with initiation of spike activity. GABA and Gly produced both monophasic and biphasic voltage responses, the latter usually requiring greater iontophoretic currents. The inversion potentials of the monophasic responses varied, but when compared, were similar for both GABA and Gly. The inversion potential of the later, depolarizing phase of biphasic responses was typically 8-10 mV depolarized relative to the hyperpolarizing phase. All GABA and Gly voltage responses were associated with increases in membrane conductance, which decayed semi-logarithmically in either one or two phases. The decay of the Gly conductance change was always more rapid than that of GABA for extrapolated peak conductance increases of the same magnitude. The clear differences in postsynaptic kinetics for these two putative inhibitory transmitters may account for the relatively shorter duration of spinal, and longer duration of supraspinal inhibitory postsynaptic potentials (ipsps), which are thought to be mediated by Gly and GABA, respectively. The results thus suggest that the differences in ipsp duration are due to differences in the kinetics of the postsynaptic conductances activated.

PB applied by microiontophoresis or diffusion either did not affect resting membrane properties or rapidly and reversibly increased membrane conductance by up to 50%. The latter effect reduced excitability, as evidenced by depression of spike genesis to constant current depolarizing pulses. The inversion potential of the PB voltage response was similar to that of the monophasic GABA response or of the hyperpolarizing phase of the biphasic GABA response. PB rapidly and reversibly depressed depolarizing Glu responses independent of its effects on resting membrane properties. Analysis of double-reciprocal plot data indicates that the antagonism of the Glu response is non-competitive. PB rapidly and reversibly prolonged the conductance change induced by GABA in 95% of cells tested, but did not affect that elicited by Gly. The increase in half decay time (T 1/2) of the GABA response averaged 67%. When the GABA conductance change decayed in two phases, the T 1/2 of both phases was prolonged. In 40% of cells examined PB enchanced the extrapolated peak conductance increase induced by GABA, while in the remaining cells PB either caused no change or depressed the peak conductance increase. PB did not alter the inversion potential of monophasic GABA responses. In those cells possessing biphasic GABA responses, PB produced a qualitative change in the voltage response, markedly enhancing the later depolarizing phase which inverted at a potential 8-10 mV depolarized relative to the unchanged inversion potential of the hyperpolarizing phase. The changes in T 1/2 and inversion potential of GABA responses were largely mimicked by increasing the magnitude of the GABA iontophoretic pulse.

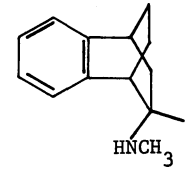
The results suggest that part of the depression of neuronal excitability in the mammalian CNS by PB derives from postsynaptic effects of the anesthetic, leading to depression of excitatory events and enhancement of GABA-mediated inhibitory events. The locus of the depressant effect of PB on Glu excitation does not appear to be at the receptor level, while the locus of the potentiating effect of PB on GABA inhibition remains to be determined.

1079 EFFECT OF CONFORMATION ON SYNAPTOSOMAL UPTAKE OF AMPHETAMINE ANALOGS. R. Martin Bartholow*, James A. Ruth*, Gary L. Grunewald*, and Charles O. Rutledge. Depts. of Pharm. and Tox. and Med. Chem., Univ. of Kansas, Lawrence, KS 66045.

The uptake of the exo (NM-X) and endo (NM-N) isomers of ^{3}H-2-methylaminobenzobicyclo[2.2.2.]octene was used to study the effect of side chain conformation of amphetamine (AM) on uptake of phenethylamines by synaptosomes. These isomers are rigid analogs in which the phenethylamine group is either in an extended (NM-X) or folded (NM-N) conformation. Synaptosomes were prepared by sucrose density gradient centrifugation from either rat hypothalamus or corpus striatum. The data are expressed as the mean±SEM of T/M ratios (dpm per mg protein/dpm per ml medium). NM-X (5μM) was accumulated in synaptosomes to a greater extent (P<.001) at 37° (T/M, 244±20) than at 0°C (T/M, 138±14). Similar results were obtained for NM-N. In synaptosomes derived from the corpus striatum the T/M ratio for NM-X was again significantly greater (P<.01) at 37° (287±22) than at 0°C (185±21). The accumulation of NM-N in corpus striatum was less (P<.02) than that of NM-X both at 37° (179±19) and at 0° (116±10). The accumulation of NM-N was significantly greater (P<.05) at 37° than at 0°. To determine whether the greater accumulation at 37° is due to neuronal uptake, the rigid analogs were incubated in the presence of desipramine (DMI, a competitive inhibitor of norepinephrine uptake) or dopamine, (DA, a competitive agonist for DA uptake sites). DMI blocked the uptake of NM-N and NM-X into hypothalamic synaptosomes, while DA blocked uptake of NM-X, but not NM-N into synaptosomes from the corpus striatum. As an index of passive diffusion across the neuronal membrane, the n-octanol: water partition coefficients were determined. The values are: NM-N 3.4, AM 2.1, NM-X .55, DA .12 and norepinephrine .08. The degree of ionization also influences the transport of drugs across lipid membranes. The pKa of these compounds range from 9.75 to 9.99, with the exception of DA, which is 10.63. In conclusion, the conformational differences between the extended and folded analogs of AM lead to different neuronal uptake properties. The neuronal membrane of the uptake system appears to prefer the extended conformation. This is particularly true in the corpus striatum. Since NM-N is more lipid soluble, it is more likely to enter by passive diffusion. The increased lipid solubility and the decreased affinity for the neuronal uptake system by NM-N may be due to the shielding of the nitrogen which is folded towards the phenyl ring. Supported by USPHS, NIH, Grants NS 12760, GM 1341, Univ. of KS, Gen. Res. Supp. and Kans. Heart Assoc.



NM-X



NM-N

1080 CATECHOLAMINE-CONTAINING FIBERS IN TISSUE CULTURES OF FETAL RAT HYPOTHALAMUS. Lucy L. Brown*, Susan C. Feldman*, (SPON: M. Bornstein) Dept. of Neurology, Albert Einstein College of Medicine, Bronx, New York 10461.

As part of a study concerned with the morphology of the hypothalamus in vitro, cultures were investigated for the presence of catecholamine (CA) cells. Explants were obtained from 20-day old fetuses; each hypothalamus was bisected along the third ventricle and each half subdivided into six fragments. Cultures were maintained for 40 days in a Maximow double-coverslip assembly in medium containing 33% human placental serum. Culture-bearing coverslips were prepared for fluorescence microscopy by immersion in buffered 2% glyoxilic acid (GA) solution (pH 7.4, 4°C) for 3 min, dried, and then heated for 7 min at 80°C. The criteria for identifying the fluorescence as CA-specific were 1) that visualization of CA fibers was dependent on reaction with glyoxilic acid; 2) that fluorescence disappeared after exposure to intense UV light; 3) that the wavelength for peak excitation shifted after exposure to HCl vapor; 4) that microspectrofluorometric determination of emission and excitation peaks of fibers were similar to those seen for CA droplets on collagen-coated coverslips.

In 17 of 19 cultures examined, green fluorescent fibers were seen. Fibers formed dense networks or bundles which extended into the outgrowth region, in general from one edge of the culture. The morphologic characteristics of single fibers were similar to those of non-terminal and pre-terminal dopaminergic axons in the adult rat; i.e., very fine smooth fibers (1-2μ) or fibers with delicate round varicosities. The cell bodies of origin could not be seen within the thick, bright yellow explant. Hippocampus, cultured under identical conditions, did not show fluorescent fibers.

In conclusion, long-term tissue culture of fetal hypothalamic fragments allows for vigorous growth of CA-containing fibers. These fibers resemble those seen in the adult rat in situ, both in terms of their structure and their arrangement in bundles. These results suggest that this system is a useful in vitro model for the development and function of CA cells in the hypothalamus.

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1081 DISTINCTION BETWEEN THE NICOTINIC RECEPTORS OF GANGLIA AND NEUROMUSCULAR JUNCTIONS BY MEANS OF SNAKE NEUROTOXINS.

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α-Bungarotoxin (α-BTX) and cobra venom have been shown to block neuromuscular transmission and to bind to the acetylcholine (Ach) receptors of the post-junctional membrane. In 1973, Green, Sytkowski, Vogel, and Nirenberg reported that ^{125}I- α-BTX bound to dissociated chick ganglion cells grown in-vitro. The present study was done to determine if these neurotoxins also block ganglionic transmission by binding to the Ach receptors of ganglia. Neither α-BTX nor cobra venom blocked responses of isolated guinea pig longitudinal muscle with adherent myenteric plexus to the nicotinic agonists nicotine (10 µM) or DMPP (10 µg/ml), to Ach (1 - 10nM), or to electrical field stimulation. The toxins also failed to affect responses of the isolated guinea pig stomach to preganglionic stimulation by way of the vagus nerves or of the vas deferens to preganglionic stimulation via the hypogastric nerves. Moreover, the toxins did not block non-adrenergic inhibitory responses of the rabbit small intestine to electrical field stimulation or nicotine. The toxins were equally ineffective against nicotinic agonists in preparations of newborn rabbit or embryonic chick intestine. No binding of rhodamine or ^{125}I-labelled α-BTX to intestinal ganglia was seen. The possibility that the toxins' failure to act was due to their inability to reach ganglionic nicotinic receptors was studied ultrastructurally in intestinal ganglia. No potential permeability barriers were found. Ganglion cells and dendrites reach the surface of the myenteric plexus and, uncovered, abut on the connective tissue space. The tracer molecules lanthanum and ruthenium red readily penetrate into the plexus reaching synaptic gaps. Exposure of intestinal preparations to hypertonic sucrose, or to calcium free medium containing EDTA, prior to addition of α-BTX, or adding α-BTX together with dimethylsulfoxide did not enable α-BTX to alter responses to nicotinic agonists. These experiments indicate that the failure of snake neurotoxins to block ganglionic transmission or to bind to ganglionic nicotinic receptors is not due to their failure to reach these receptors. Ganglionic Ach receptors must therefore be different from those of the neuromuscular junction.

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1082 THE DEPOLARIZATION BY GABA OF MAMMALIAN PRIMARY AFFERENT GANGLION CELLS: A STUDY OF DESENSITIZATION IN VIVO. M. Deschênes*, Y. Lamour* and P. Feltz* (SPON: M. Steriade). Laboratoire de Physiologie des Centres Nerveux, Université Pierre et Marie Curie, Paris, France.

The GABA receptors of dorsal root ganglion neurones of the rat were studied in vivo with intracellular recording of membrane potential, and measurements of membrane conductance and of extracellular potassium activity (Feltz and Raminsky, Neuropharmacol. 13: 553, 1974). Local application of GABA either by addition to the superfusion medium or by micro-iontophoresis led to a biphasic depolarizing response. The response showed a transient peak undergoing rapid desensitization and a second dose-dependent plateau of lower amplitude - at the most 20% of the peak amplitude -. In contrast to the peak response, the later low-level sustained depolarization lasted for as long as GABA was present in the medium. The desensitization of the membrane voltage response induced by GABA seems to run parallel to a desensitization of the chloride conductance change underlying the response to the amino-acid. The time course of desensitization was indicated by the transient responses to iontophoretic pulses of GABA (threshold dose: 50-200 nA, 0.5-1 sec) applied while ganglion was being superfused with 10^{-3} - 10^{-4} M.GABA in Ringer of conventional ionic contents. By means of these tests it was found that GABA-induced depolarization was not due to a change in the equilibrium potentials of the ions involved since the transient responses were not reinstated to their initial magnitude by hyperpolarizing the membrane. Nevertheless, when GABA was present in the medium the extracellular K^+ concentration, as sensed by K^+ sensitive microelectrode, underwent a prolonged increase. This was further suggested by a reduction in the hyperpolarizing after-potentials of ganglion spikes. These changes in K^+ might further participate in the low-level sustained depolarization resistent to desensitization. Supported by CRSQ (Canada) and by DGRST (France).

1083 EFFECT OF ADENOSINE 5'-MONOPHOSPHATE ON THE MEMBRANE POTENTIAL OF CORTICAL NEURONES IN THE RAT. J.P. Edstrom* and J.W. Phillis. (SPON: J.S. Richardson). Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.

There have been several reports documenting the depressant actions of adenosine, along with its derivatives and analogues, on both spontaneously active and glutamate driven cortical neurones (Phillis and Kostopoulos, Can. J. Phys. Pharm. 52: 1226, 1974; Phillis, Kostopoulos and Limacher, Eur. J. Pharm. 30: 125, 1975; and Kostopoulos, Limacher and Phillis, Brain Res. 88: 162, 1975). A possible interpretation of these findings is that adenosine, or one of its derivatives, acts as a synaptic transmitter similar to the role proposed by Burnstock (Pharm. Rev. 24: 509, 1972) for adenosine triphosphate in smooth muscles. To explore this possibility the membrane potential of cortical neurones was recorded while adenosine 5'-monophosphate (AMP) was extracellularly iontophoresed near the cell. Male Sprague Dawley rats anaesthetized with methoxyflurane and nitrous oxide were used in these experiments. The intracellular microelectrodes were fabricated from pyrex capillary tubes, filled with 5 M potassium acetate, sharpened on a Brown type micro-pipette beveler and had resistances of between 20-50 MΩ after beveling. A Mentor model N-950 preamplifier was used with a silver-silver chloride electrode implanted in the rat's neck.

The results consistently show that the major effect of AMP is a reduction in the amplitude of the spontaneously occurring excitatory postsynaptic potentials (EPSP's). There is also a hyperpolarization but this may be due to the withdrawal of the EPSP's and not a direct AMP effect. There is no substantial change in the input impedance of the cell measured by the voltage change across the membrane in response to square hyper- or de-polarizing intracellular current pulses. These facts suggest that the site of AMP's action is presynaptic. It has been shown at the neuromuscular junction (Ginsborg and Hirst, J. Physiol. 224: 629, 1972) that adenosine can decrease the quantal content of the end-plate potential. This may also be true in the cerebral cortex. There are two other possibilities. The first is that the adenosine receptor may be localized far out on the dendrites so that although the AMP may block transmission from the dendritic extremities, its direct effect may not be observable from the soma where we presume the recording electrode is located. The second is that the AMP may be blocking the synaptic receptors so that normal presynaptic events are not received postsynaptically. Presently the data does not discriminate among these three possibilities.

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1084 SOMATOSTATIN CONTAINING PATHWAYS IN THE NERVOUS SYSTEM. R. Elde, T. Hökfelt*, O. Johansson*, S. Efendić* and R. Luft*. Dept. Anat., Univ. Minnesota, Mpls, MN 55455; Depts. Histol. and Endocrinol. & Metab., Karolinska Institute, S-104 01 Stockholm, Sweden.

In previous studies we have reported the immunohistochemical localization of somatostatin (SOM) in various regions of the CNS and PNS (Acta endocrinol. 80, Suppl.200, 1975; Am. J. Anat. 144:541, 1975; Neurosci. Letts. 1:231, 1975). Combining radioimmunoassay with immunohistochemical techniques, the present study focuses on the projections and terminal areas of the two identified populations of SOM perikarya - those of the dorsal root ganglia and those of the anterior hypothalamic periventricular nucleus.

Radioimmunoassay of acid-alcohol extracts of segments of rat spinal cords revealed that the lumbar cord contained 206±6 pg SOM/mg wet weight whereas cervical-thoracic segments contained 132±13 pg SOM/mg wet weight (n=3). This confirms the qualitative impression previously obtained by immunohistochemistry. Dorsal root transection markedly reduced the content of dorsal horn SOM, whereas no changes were observed after spinal cord transection. These results support our contention that a subpopulation of primary sensory neurons contain SOM. In further studies we have shown that this SOM system is distinct from, and yet in many aspects parallel to, a primary afferent system containing substance P.

Immunohistochemical studies after bilateral electrolytic destruction of the periventricular nucleus demonstrated the complete disappearance of SOM from the median eminence. On adjacent sections, thyrotropin releasing hormone was also absent from the median eminence but gonadotropin releaseing hormone appeared to be present in normal quantities. In spite of the destruction of all known hypothalamic SOM perikarya, the dense SOM innervation of the ventromedial and arcuate nucleus remained intact. These results suggest that there may be more SOM systems than previously characterized. Furthermore, the results also suggest that the possibility exists that hypothalamic nuclei innervated by SOM may be independent of the SOM neurosecretory pathway to the median eminence. (Supported by a NINCDS fellowship (NS 05047-01) and Swedish Medical Research Council).

1085 BIOGENIC ALDEHYDES IN THE CONTROL OF DEPRESSANT STATES AND BIOGENIC AMINES IN THE CONTROL OF EXCITATORY STATES. Aaron Feldstein, Rutgers University, Center of Alcohol Studies, New Brunswick, NJ 08903 and Vincent DaForno*.

Depressant states may be controlled in part by biogenic aldehydes in general and indoleacetaldehydes in particular, e.g. 5-hydroxyindoleacetaldehyde (5-HIAAld), and excitatory states may be controlled in part by biogenic amines in general and catecholamines in particular, e.g. norepinephrine, dopamine. The concepts of B.B. Brodie relevant to the counterposition of excitatory, ergotrophic systems mediated by norepinephrine and depressant, trophotrophic systems mediated by serotonin appear to be esentially correct if we take into account the depressant properties of 5-HIAAld, the metabolite of serotonin.

In many species and CNS structures, the normal MAO concentration may favor a low serotonin/5-HIAAld ratio in serotonergic neuronal systems with predominence of depressant states. In catecholamine neuronal systems, the normal MAO concentration may favor a high amine/aldehyde ratio, with predominence of excitatory states. Any perturbation of these two normally antagonistic systems in brain, physiologically-, pathologically-, or drug-induced, which tends to increase amine or decrease aldehyde bioavailability at their respective receptor sites may shift the balance in favor of excitatory states. A decrease in amine or increase in aldehyde bioavailability may favor depressant states.

Pharmacological procedures with a potential for increasing exogenous brain aldehydes and activating aldehyde receptor sites induced depressant states, i.e. administration of tryptophan, 5-hydroxytryptophan (5-HTP), serotonin, 5-HIAAld, 5-hydroxytryptophol (5-HTOL). Procedures with a potential for increasing endogenous biogenic aldehydes also induced depressant states, i.e. administration of pyrazoles (inhibitors of alcohol dehydrogenase), barbiturates, chlorpromazine (inhibitors of aldehyde reductase) and disulfiram, etc. (inhibitors of aldehyde dehydrogenase).

Data, using the thermoregulatory system of the rat, implicates 5-HIAAld as a depressant, hypothermic substrate and serotonin *per se* as an excitatory, hyperthermic substrate. Serotonin and 5-HTP, precursors of 5-HIAAld, administered i.p., were markedly hypothermic in the untreated rat and less hypothermic, even hyperthermic, in the rat pretreated with phenelzine to increase serotonin and decrease 5-HIAAld. Tryptamine and tryptophol which are converted to indoleacetaldehyde (IAAld), an analog of 5-HIAAld, were hypothermic after i.p. administration. 4-Methylpyrazole (4-MPZ), an alcohol dehydrogenase inhibitor, which blocked the interconversions of 5-HIAAld and 5-HTOL, also induced hypothermia. 4-MPZ in low dose potentiated the tryptophol-induced hypothermia, probably due to inhibition of liver alcohol dehydrogenase. In high dose, 4-MPZ blocked the tryptophol-induced hypothermia, possibly due to inhibition of brain alcohol dehydrogenase which would increase tryptophol and decrease IAAld. Pharmacological evidence also supports the assignment of a role for biogenic aldehydes in other depressant states, i.e. decreased motor activity, sedation and sleep, anticonvulsant action and inhibition of ovulation.

(The laboratory work was done at the Worcester Foundation for Exper. Biol. with support from a USPHS grant, MH-13540).

1086 PHYSIOLOGICAL CONTROL OF BRAIN CATECHOLAMINE SYNTHESIS; EFFECT OF BRAIN TYROSINE CONCENTRATION. Candace J. Gibson* and Richard J. Wurtman (SPON: L. D. Lytle). Laboratory of Neuroendocrine Regulation, MIT, Cambridge, MA 02139.

We have previously shown that treatments which raise or lower brain tyrosine in rats cause parallel changes in the rate at which brain accumulates DOPA, following L-amino acid decarboxylase inhibition by RO4-4602 (Wurtman et al., *Science* 185:183, 1974). We now report that: a) this relationship is linear over a wide range of brain tyrosine concentrations; b) none of the neutral amino acids that we used to modify brain tyrosine level acts as a direct inhibitor of tyrosine hydroxylase; and c) the consumption of a single high-protein meal (i.e., a normal event) raises both brain tyrosine level and catechol synthesis.

The neutral amino acid drug, p-chlorophenylalanine (pCPA), inhibits tyrosine uptake into brain and was used to produce animals with a wide range of brain tyrosine concentrations. After its administration, brain DOPA accumulation paralleled tyrosine levels ($r = 0.78$; $p < 0.001$). Tyrosine hydroxylase activity was not inhibited by these pCPA doses in vivo (and was only slightly inhibited at very high pCPA concentrations in vitro). These findings suggest that pCPA's effect on DOPA accumulation derives from changes in the availability of the tyrosine substrate rather than from changes in enzyme activity.

Similarly, the neutral amino acids leucine, valine, and isoleucine (which we have used previously to modify brain tyrosine concentration and, thereby, catechol synthesis) had no effect on tyrosine hydroxylase activity in vitro up to concentrations of 1 mM in the assay medium (Waymire et al., *Anal. Biochem.* 43:588, 1971). One hour after the intraperitoneal injection of either valine or isoleucine (100 mg/kg), brain valine levels increased to only 0.2 mM, and brain isoleucine to 0.07 mM. Hence, it is unlikely that these amino acids directly affect tyrosine hydroxylase activity in vivo.

The ingestion of a single meal containing 40% casein both elevates brain tyrosine levels (by 60-70% after 90 min) and accelerates brain catechol synthesis (by 60%). These observations suggest that the availability of tyrosine to the brain may be one of the factors normally controlling brain catecholamine synthesis.

1087 COMPARTMENTATION OF GLUCOSE METABOLISM IN RELATION TO THE TURNOVER OF ACETYLCHOLINE. G.E. Gibson*, L. Graue1*, J.P. Blass and D.J. Jenden.
UCLA Medical School, L.A., Ca. 90024.

The turnover of acetylcholine (ACh) in vivo from [^{3}H]choline (1), [$^{2}H_4$]-choline (2), and [^{14}C]phosphorylcholine (3) has been shown by others. We have now determined the turnover of ACh in vivo by labelling the acetyl-moiety of ACh with [U-^{14}C]glucose. Mice were injected intravenously with [U-^{14}C]glucose (30-100 μCi/30 g; 230 μCi/μmole). At various times after injection (1,2,5,10,15,30 and 60 min) the mice were sacrificed by micro-wave irradiation. The molar specific activity (DPM/nanatom) of glucose, pyruvate and ACh were determined by enzymatic methods and GC-mass spectro-metry. The injection of [U-^{14}C]glucose did not alter the concentration of glucose, pyruvate or ACh. The amount of ^{14}C incorporated into all frac-tions was proportional to the amount of ^{14}C injected when 1,2 or 4 μCi/g of [U-^{14}C]glucose were injected. Incorporation of [U-^{14}C]glucose into ACh peaked 5 min after injection of [U-^{14}C]glucose. At that time the following molar specific activities (DPM/nanatom) were observed: glucose, 70±2.7; ACh, 57.5±11.51; pyruvate, 24.3±1.7. At all time points tested the molar specific activity of ACh was higher than the molar specific activity of pyruvate. Although the molar specific activity of ACh was equal to that of glucose by 10 min, the molar specific activity of pyruvate was not equal to that of glucose until 60 min. These results indicate to us that a small rapidly turning over pool of glucose within the nerve ending is be-ing utilized for ACh synthesis and that total brain pyruvate is not in equilibrium with this pool.

A turnover rate of 3.0 nanomoles of ACh per min per 100 mg protein can be calculated from this data using the molar specific activity of glucose as that of the precursor pool. A turnover rate of 8.6 nanomoles of ACh per min per 100 mg protein can be calculated using the molar specific ac-tivity of pyruvate as that of the precursor pool. These turnover rates agree well with the turnover rates calculated using [$^{2}H_4$]choline as a pre-cursor [1.4 and 7.9 nanomoles per g per min (2)]. The agreement is par-ticularly striking if one considers the many assumptions involved in both methods of determining turnover.

In vitro, when brain slices are incubated in a modified Krebs-Ringer buffer containing 31 mM-K^{+}, the molar specific activity of ACh in the supernatant is higher than the molar specific activity of pyruvate of the supernatant. These results are also consistent with a small rapidly turn-ing over pool of glucose, within the nerve endings, which labels acetyl-choline.

We have previously shown that there is a close relation between ACh synthesis and carbohydrate metabolism (4,5,6). Thus decreased pyruvate or glucose oxidation leads to decreased ACh synthesis. This is true even though 100 times as much glucose or pyruvate goes to CO_2 than is converted to ACh. Synthesis of a physiologically important pool of ACh is more sen-sitive than ATP or the adenylate energy charge to hypoxia, and pretreat-ment with the cholinesterase inhibitor, physostigmine, protected animals against seizures induced by hypoxia (5). Dolivo (7) has shown that syn-aptic transmission is more sensitive to decreased oxygen or glucose than is axonal conduction. The compartmentation of glucose shown in this com-munication helps to explain these previous findings.

References: (1) Schuberth et al. (1969) J. Neurochem. 16, 695-700. (2) Jenden et al. (1974) Life Sci. 14, 55-63. (3) Racagni et al. (1976) J. Pharm. Ex. Ther. 196, 323-332. (4) Gibson et al. (1975) Biochem. J. 148, 17-23. (5) Gibson et al. J. Neurochem. 27 (in press). (6) Gibson et al. (1976) J. Neurochem. 26, 1073-1079. (7) Dolivo (1974) Fed. Proc. 33, 1043-1048.

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1088 MONOAMINE OXIDASE, CATECHOL-O-METHYL TRANSFERASE, LDH AND γ-AMINOBUTYRIC ACID IN HUMAN VENTRICULAR FLUID FOLLOWING BRAIN INJURY. R.G. Grossman, C. Beyer*, G. Shannon*, P. J. Kelly, and B. Haber. Division of Neurosurgery, Dept. of Surgery and The Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, Texas 77550.

The purpose of this study was to determine whether monoamine oxidase (MAO), catechol-o-methyl transferase (COMT) and γ-aminobutyric acid (GABA) are present in significant amounts in human ventricular fluid (VF) in the presence and absence of traumatic head injury (HI). Lactic dehydrogenase (LDH), and its isozymes, were also measured in both VF and serum as a cytoplasmic marker indicative of cellular damage. Ventricular fluids were obtained via catheters placed for intracranial pressure monitoring and relief of elevated intraventricular pressure in HI, and during stereotactic surgery for the relief of pain and dystonia. The contribution of cellular components of blood to VF and serum values was excluded by discarding all hemolized samples and rapid centrifugation of the fluids included in these studies. MAO was measured using a radiometric assay; on the basis of substrate preference, we believe that VF MAO is type A, and therefore its presence in VF represents leakage of CNS cell contents into CSF. In most, but not all VF from head injured patients the MAO is elevated. COMT was measured by a radiometric procedure; measurable amounts of the enzyme were present in both control and head injury fluids. GABA was measured by the fluorimetric-enzymatic method and verified unequivocally by gas chromatography-mass spectrometry. The concentrations of GABA in both control and head injury ventricular fluids ranged from 0.27 to 1.44 μM/L. In the limited number of VF from patients with movement disorders studied, the GABA values were near the lower limit of the range. LDH values were elevated in both serum and VF of head injury patients. All of the LDH isoenzymes were elevated, especially isoenzymes 1, 2 and 3. The elevation of isoenzymes 1, 2 and 3 also suggests cellular damage of CNS origin. The use of these assays to study the extent of brain cell damage in head injury is under investigation.

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1089 LOWERING OF NORMAL BODY TEMPERATURE AND BLOCKADE OF FEBRILE RESPONSES BY CENTRAL ADMINISTRATION OF TAURINE. William S. Harris* and J.M. Lipton. Depts. Psychiat., Physiol. and Neurol., University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235.

Taurine (2-aminoethanesulfonic acid), an amino acid found in high concentration in the brain, has been shown to produce hypothermia when given peripherally and intracerebroventricularly (i.c.v.) to mice (Hruska *et al.*, 1976) and i.c.v. to rats (Sgaragli and Pavan, 1972). The purpose of the present experiments was to test the central antipyretic capacity of taurine and to elucidate the mechanism through which this substance causes hypothermia. In rabbits resting in a 23°C environment, 1-6 mg taurine i.c.v. produced vasodilitation and dose-related decreases in rectal temperature (T_{re}). In a cold environment (10°C), 0.5 and 1 mg doses caused hypothermia and 0.1 mg did not alter T_{re}. In a hot environment (30°C), taurine (0.1-2 mg) had no effect on T_{re}. A 0.5 mg bolus of taurine followed by slow infusion (0.01-0.1 mg/min, i.c.v.) blocked the febrile response to i.v. *S. typhosa* endotoxin (1 µg/kg). Control infusions of taurine at the same rates in the same rabbits, when they were afebrile, had little effect on T_{re}. Hyperthermia caused by i.c.v. injections of PGE_1 (2 µg), a substance which has been implicated in fever mediation, was blocked by a high dose (5 mg) of taurine and reduced by 0.5 mg. Similar doses of taurine had no effect on amphetamine-induced hyperthermia (2 mg/kg; i.v.). Bilateral injections of taurine (0.1 mg) into the primary temperature control in the preoptic/anterior hypothalamic region, at sites where *S. typhosa* endotoxin (1 µg) caused long-lasting fevers, had no effect on T_{re}. Similar injections into the reticular substance of the medulla oblongata, in the region believed to contain a secondary temperature control, also did not alter body temperature. These data suggest that taurine does not produce hypothermia and antipyresis by altering the set point of central temperature controls. Rather this amino acid appears to inhibit neuronal activity in effector pathways which control vasomotor tone, shivering and the level of arousal.

1090 ELEVATION OF BRAIN AND ADRENAL ACETYLCHOLINE LEVELS AND OF ADRENAL TYROSINE HYDROXYLASE ACTIVITY FOLLOWING ADMINISTRATION OF CHOLINE VIA STOMACH TUBE. M.J. Hirsch*, I. Ulus*, R.J. Wurtman (SPON: F. Worden). Massachusetts Institute of Technology, Cambridge, MA 02139.

Brain choline and acetylcholine (ACh) levels increase for 40-60 minutes after rats receive choline intraperitoneally,(Cohen and Wurtman, *Life Sci.*16:1095,1975). We now observe that prolonged increases in both compounds are obtained if a choline chloride (ChCl) solution is administered by stomach tube.

Among unfasted 200 gm. rats killed 5 hours after receiving 2.5 gm/kg of ChCl (and killed by microwave beam focussed on the head) choline concentrations were significantly elevated in the cortex (258% rise,$P<.001$) and the caudate (110% rise,$P<.01$); similar changes were observed after 8 hours. ACh concentrations were elevated (by 58% and 57%) after 5 hours, but decreased to control and to 86% of control values, respectively, after 8 hours. This decrease in the presence of persistantly elevated choline concentrations may infer the operation of a feedback mechanism coupled to ACh synthesis or degradation.

Choline intubation (2.5 gm/kg) also elevated adrenal choline and ACh levels; these peak within 1-2 hours, and approach control values by 16 hours. In contrast, tyrosine hydroxylase activity becomes significantly elevated 24 hours after choline administration.

(hours)	Cont.	1	2	4	8	16	24
Adr. Choline (μm/gm)	0.40 ±0.20	1.65$^{\Phi}$ ±0.30	1.84** ±0.53	0.80 ±0.12	0.86 ±0.11	0.58 ±0.06	--
Adr. ACh (nm/gm)	29.70 ±2.91	77.53* ±16.45	137.47$^{\Phi}$ ±19.62	93.50* ±29.79	139.27** ±43.27	39.43 ±8.18	--
Tyrosine Hydrox. (nm CO_2/hr/ mg. prot.)	3.37 ±0.26	--	3.14 ±0.35	3.67 ±0.23	3.88 ±0.13	3.63 ±0.28	4.45$^{\Phi}$ ±0.22

$\Phi P<.01$ compared to control values.
$*P<.02$ compared to control values.
$**P<.05$ compared to control values.

This suggests that: 1)precursor-induced increases in neuronal ACh levels are associated with parallel changes in ACh release, and, 2) in the adrenal medulla, this increase in ACh release produces post-synaptic effects, i.e., an increase in tyrosine hydroxylase activity, probably resulting from enzyme induction.

1091 CHANGING SYNAPTIC VESICLE CYTOCHEMISTRY IN CULTURED SYMPATHETIC NEURONS.
Mary Johnson, David Ross*, Marta Meyers* and Richard Bunge. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

Prior studies have shown that cultures of dissociated principal neurons from the superior cervical ganglion of perinatal rats exhibit certain expected adrenergic properties. In addition, these cultured neurons were found, unexpectedly, to form synaptic contacts with each other and to interact physiologically by a nicotinic-cholinergic mechanism. Morphological studies have reported that these synaptic contacts contain dense core vesicles. The question is thus raised whether the cholinergic mechanisms derive from one population of neurons and the adrenergic from another, or whether one cell type is responsible for both. In the present study we address this question by analyzing the types of vesicles present in synaptic contacts after various periods in culture. Concomitant studies on sister cultures revealed increasing levels of choline acetyltransferase activity after three weeks in culture, as well as the onset and increasing complexity of cholinergic interactions (Johnson et. al., Nature, in press).

Dissociated neurons from perinatal rats were established in culture using a method modified from Bray (1970). They were maintained with supporting cells for two months in 5% CO_2 using a medium containing serum, embryo extract, and NGF. Between 1 to 8 weeks, cultures were incubated at weekly intervals in freshly prepared 10^{-5}M norepinephrine (pH 7.2-7.3) and fixed in 3% $KMnO_4$ with subsequent embedding in epon-araldite. Twenty-five consecutive contacts on neuronal somata and proximal dendrites were photographed for each time period. Prints were made at a standard magnification and coded for blind analysis. The vesicles measuring 40-70 nm in each ending were classified and counted by 3 observers. Subsequent analysis revealed that at one week in culture the predominant vesicle type in all endings contained dense cores, none containing more than 30% clear vesicles. As early as two weeks in culture a shift toward endings containing predominantly clear vesicles was observed. By eight weeks in culture 21 of the 25 endings observed contained greater than 50% clear vesicles. An analysis of endings at intermediate times showed a mixed population of dense core and clear vesicles. The morphology of the contacts was that characteristically seen in adrenergic systems. Not infrequently profiles consisting of two to three connected varicosities containing the same predominate vesicle type were seen. As expected, the postsynaptic densities were not prominent in $KMnO_4$ fixed material but neither were they striking when aldehyde was used for fixation. Also, no definite clustering of the vesicles toward the contact site was observed. We conclude from these data that the cholinergic synapses which develop between rat superior cervical ganglion neurons in our culture system do not derive from differentiation of a subpopulation of cholinergic neurons within a larger group that is predominately adrenergic. The presence of synaptic endings with approximately equal numbers of dense core and clear vesicles during the period when cholinergic interactions appear and choline acetyltransferase activity rises suggests that cholinergic and adrenergic mechanisms are expressed within the same neuron. (Supported by NIH grants NSO 9923 and NS 11888).

1092 IN VIVO TURNOVER OF ^{3}H-5-HYDROXYTRYPTAMINE TO ^{3}H-5-HYDROXYINDOLEACETIC ACID AND THE ^{3}H-5-METHOXYINDOLES IN NON-DEPRIVED AND 24 HR FOOD DEPRIVED RATS. K. M. Kantak*, M. J. Wayner, A. Sved* and H. A. Tilson*. Brain Res. Lab., Syracuse Univ., Syracuse, NY 13210. (SPON: A. Kastin)

Serotonin turnover in the right lateral hypothalamus was analysed in non-deprived and 24 hr food deprived rats. This region was infused with 0.5 µCi of ^{3}H-5-hydroxytryptamine 1 hr prior to push-pull perfusion with physiological bacteriostatic saline. There were no significant differences between the ^{3}H-washout curves for both groups of rats. The percentage of nCi/µCi of radioactivity was analysed by thin layer chromatography for 5-hydroxytryptamine (5-HT); 5-hydroxyindoleacetic acid (5-HIAA); 5-methoxytryptamine (5-MT); 5-methoxytryptophol (5-MTPhol); and 5-methoxyindoleacetic acid (5-MIAA). These compounds were analysed for 3 successive samples corresponding to 75-90 min post infusion. Turnover of 5-HT to all four metabolites was detected in both groups of rats. However, 5-HT turnover was different in non-deprived and 24 hr food deprived rats. For the total 15 min period, there was significantly more 5-HIAA and 5-MT formed in the 24 hr food deprived rats than in the non-deprived rats. No differences were found in the percentages of radioactivity for 5-HT, 5-MIAA and 5-MTPhol between groups. When 5-HT turnover for each group was examined in individual successive samples, differences occurred over time as well. The percentage of radioactivity for 5-HT in non-deprived rats decreased in the 80-85 min period and then returned to the 75-80 min level during the 85-90 min period. The percentages of radioactivity for all four metabolites in this group did not change over time. In the 24 hr food deprived rats the percentage of radioactivity for 5-HT did not change over time. The percentage of radioactivity for 5-MT in this group increased over time, with the highest percentage of radioactivity occurring 85-90 min post infusion. The percentages of radioactivity for the other three metabolites did not change over time in this group. These data show larger amounts of two metabolites of 5-HT formed in the lateral hypothalamus of 24 hr food deprived rats. This may indicate a faster 5-HT turnover rate in the lateral hypothalamus of 24 hr food deprived rats than in non-deprived rats.

1093 RESPONSIVENESS OF DOPAMINE-STIMULATED ADENYLATE CYCLASE OF HUMAN, MONKEY, CALF AND CEPHALOPOD RETINA TO AGONISTS AND TO NEUROLEPTIC DRUGS AND COMPARISON WITH BRAIN: EVIDENCE FOR DIFFERENT CLASSES OF CENTRAL DOPAMINE RECEPTORS AND FOR DEVELOPMENTAL AND EVOLUTIONARY CHANGES. Maynard H. Makman*, B. Dvorkin* and Sara G. Horowitz*. (SPON: W. T. Norton). Albert Einstein Col. Med., Bronx, N. Y. 10461.

Dopamine (DA)-stimulated adenylate cyclase activity (AC) is present in retina of all mammalian species examined thus far as well as in retina of the marine invertebrate, Octopus bimaculatus (Makman et al, Adv. Cyclic Nucl. Res., 5, 661,1975) and the influence of a number of neuroleptic drugs on DA-sensitive AC of calf retina has been examined (Brown and Makman, J. Neurochem., 21, 477, 1973). We now report the presence and properties of DA-sensitive AC of human retina as well as a more detailed study of the response to neuroleptic drug-antagonists and DA-agonists in Cebus and rhesus monkey and octopus retina. Human retina contains DA-sensitive AC with pharmacological characteristics similar to that of monkey retina. Only primate DA-stimulated AC is activated significantly by isoproterenol. Norepinephrine activates all the mammalian systems but not the highly specific octopus system which is also insensitive to apomorphine and the DA-agonist 1-(3,4-dihydroxylbenzyl)-4-(2-pyrimidinyl)-piperazine (S584). Rhesus monkey retina 40 hours - 6 days after birth, in contrast to adult retina, has little response to isoproterenol, S584 or apomorphine. In the invertebrate system pimozide is more than 100X as potent as fluphenazine or haloperidol, with complete blockade of 10^{-5} DA by 10^{-9} M pimozide. Sensitivities of human and octopus retina to fluphenazine were similar but pimozide at 10^{-5} M blocked 10^{-5} M DA only partially in human retina. The DA-AC of primate retina resembled in general that of primate brain caudate nucleus. Thus the relative sensitivity of both to haloperidol was less than that obtained for monkey anterior limbic and frontal cortex DA-AC. However, in contrast to both caudate and anterior limbic cortex, primate retina was strikingly sensitive to a drug known to have visual side effects in patients, thioridazine (close to fluphazine in potency), and relatively insensitive to clozapine. Hence, species and regional differences as well as developmental changes in DA-receptors appear to exist. The response of DA-AC to neuroleptic drugs may be a particularly useful means for elucidating these differences or changes.

1094 CHOLINE ACETYLTRANSFERASE IN CULTURES OF RAT SUPERIOR CERVICAL GANGLION.
David Ross* and Richard P. Bunge. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

Previous studies of rat autonomic neurons in culture (Chun & Patterson, 1974) and experimental embryological studies of autonomic ganglion chick-quail chimeras (LeDouarin & Teillet, 1974) suggest the type and amount of neurotransmitter production can be substantially influenced by the environment of the neuron. We have determined changes in choline acetyltransferase (ChAc) activity in cultures of neurons from rat superior cervical ganglion (SCG) in relation to (a) the length of time in culture, (b) the cocultivation of target cells known to be responsive to acetylcholine (ACh) (atrium) or to norepinephrine (fat), (c) neuronal density in culture, (d) receptor site blockade, (e) the cocultivation of either Schwann cells or periosteal fibroblasts or both, and (f) varying media components.

The adrenergic neuron of the SCG in vivo provides ACh receptors for preganglionic nerve fibers from spinal cord; in dissociated cell cultures contacts are formed among the cultured neurons, thus exposing their endings to the ACh receptor of adjacent neurons.

The increase in ChAc specific activity in SCG explants (2½ fold) after 30 days in culture was doubled when atrium was included as target and was reduced when fat was included. The average ChAc activity per neuron in cultures containing only isolated dispersed neurons of the SCG (SCGN) increased 1000 fold between 1 and 8 weeks, during which time dopa decarboxylase (DDC) activity increased 20 fold and acetylcholinesterase activity 30 to 180 fold. ChAc activity in purely neuronal SCGN cultures was less than half the activity in SCGN cultures of comparable age set out and grown with mixed supporting cells. The activity in purely neuronal SCGN cultures was doubled by the addition of either Schwann cells or periosteal fibroblasts. The average ChAc activity per neuron increased with increasing neuronal density in SCGN cultures (without supporting cells) while the average DDC activity in the same cultures decreased. The development of ChAc activity was reduced by 60% and DDC by 50% when 0.2 mM hexamethonium (HMX) was included in the medium throughout the experiment; ChAc activity was increased 2.2 fold and DDC activity was unchanged when HMX was added after 2 weeks in culture. Cultures of SCGN without supporting cells grown in various media conditions indicate that both soluble media components and CO_2 are important for expression of ChAc activity.

These experiments indicate that the cholinergic receptor and soluble factors in the media and/or supporting cells are involved in the stimulation of ChAc synthesis. Based on the observations above, our present experimentation is designed to test this basic hypothesis: Exposure to the receptor for a specific neurotransmitter is responsible for induction of the enzyme that synthesizes the appropriate transmitter. Subsequent major adjustments of these enzyme levels occur in response to a variety of environmental influences. (Supported by NIH Research grants NSO 9923 and NS 11888 and Fellowship NS 01601).

1095 CHARACTERIZATION OF 6-HYDROXYDOPAMINE-INDUCED NEUROCHEMICAL ALTERATIONS IN THE CATECHOLAMINE CONTAINING NEURONS OF CNS. Richard H. Schmidt*, Elizabeth Lewis* and Ranbir K. Bhatnagar. Dept. of Pharmacology, University of Iowa, Iowa City, IA. 52242.

Systemic injections of 6-hydroxydopamine (6-OHDA) into neonatal rats before a critical period of about 7 postnatal days results in profound alteration of the development of the central noradrenergic neuronal system. Sachs and coworkers (J. Neurochem. 22:419) on the basis of studies with ^{3}H-norepinephrine uptake, norepinephrine assay, and fluorescence histochemistry reported that such treatment results in the permanent degeneration of the cortical norepinephrine fibers but a concomitant hypertrophy or increased outgrowth of norepinephrine fibers in the pons and medulla. We have followed up these observations with determinations of the changes induced in some additional neurochemical parameters in an effort to better characterize these alterations.

Neonatal rats were injected on postnatal days 1, 2 and 3 with either 100 mg/kg 6-OHDA s.c., or vehicle. Various regions of the CNS were examined between 10 and 63 days of age. At all ages and in all areas examined no changes were found in dopamine content. Norepinephrine was almost completely depleted in the telencephalon and in parietal cortex, and was nearly doubled in the brain stem (medulla, pons and mesencephalon) at all ages. A significant reduction in tyrosine hydroxylase activity occurred, however, only in parietal cortex. The tyrosine hydroxylase activity in the telencephalon and brain stem was unaltered. Furthermore, Vmax and Km of tyrosine hydroxylase, solubilized with Triton-X 100, with respect to tyrosine did not differ from controls in these areas. Unilateral electrolytic lesions in the area of locus coeruleus of adult rats treated neonatally with 6-OHDA reduced telencephalic tyrosine hydroxylase activity by the same amount as in similarly lesioned vehicle-treated controls. In contrast, these lesions resulted in a reduction of telencephalic dopamine content in the 6-OHDA-treated group but not in the control group. Dopamine-β-hydroxylase activity did correlate with the changes seen in norepinephrine content after 6-OHDA administration.

Preliminary studies have shown that at 3 days of age telencephalic tyrosine hydroxylase activity is reduced but has begun to recover toward control levels at 6 days of age with no recovery of norepinephrine. We believe that these data suggest that dopamine containing fibers, possibly from the region of locus coeruleus, regenerate or collateralize into telencephalic but not cortical sites vacated by norepinephrine nerve terminals. Alternatively, complete degeneration of telencephalic norepinephrine nerve terminals does not occur with the 6-OHDA treatment.
(Supported by USPHS grant NS-12121.)

1096 EFFECT OF GUANETHIDINE ON DEVELOPING CULTURES OF DISSOCIATED RAT SUPERIOR CERVICAL GANGLION NEURONS. E. Wakshull*, Mary Johnson, H. Burton (SPON: A.L. Pearlman). Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

Recent observations on dissociated cultures of rat superior cervical ganglion neurons (SCGN) have demonstrated the existance of cholinergic synapses between these cells (O'Lague et al., PNAS 71: 2602, 1974; Ko et al., Br.Res. 1976, in press). Are these normally adrenergic neurons induced by an environmental factor in our culture system to become cholinergic, or is there a subpopulation of cholinergic sympathetic ganglion cells forming the observed synaptic connections? The cytotoxic drug guanethidine has been shown to be specific for adrenergic sympathetic neurons (Burnstock, et al., B.J.P.43: 295, 1971) and has been shown to destroy sympathetic nerve cells in vitro (Johnson & Aloe, Br.Res.81:519, 1974). We have used guanethidine as an assay for the "adrenergicity" of our SCGN as they develop in vitro.

Trypsin dissociated ganglion cells from 21 day rat fetuses were grown in standard media (sm) consisting of Earle's MEM, 25% human adult serum, 10% chick embryo extract, 600 mg% glucose, 1.4 mM glutamine, 1-10 units NGF. The pH was maintained by a CO_2 atmosphere at 7.3. Due to the pH sensitivity of the guanethidine effect seen in vitro, test media (tm) contained 25 mM N'-2-hydroxyethylpiperizine-N'-2 propane sulfonic acid as buffer and was kept at pH 8.00. A stock guanethidine solution was made up fresh before each application and added to tm at 25µg/ml. Control cultures were fed the same media without the drug. Cultures were fed once with the test media then returned 48 hrs later to sm.

Cell counts showed that the higher pH did not effect the viability of the control cultures, whereas the guanethidine treated cultures had up to 90-95% cell loss when treated within the first two weeks in vitro. The first signs of toxicity were beading up and fractionation of all neuritic processes within the first 36-48 hrs, followed by granulation of the somal cytoplasm as seen in the light microscope and, after a few days, cell death. Cultures treated after 4-5 weeks showed some somal granularity but recovered upon return to sm, and retained their normal electrical excitability. Little or no cell loss was seen in these cultures.

Intracellular recordings were obtained simultaneously from two SCGN grown in a comparable culture series to those used for the guanethidine studies. The results show that hexamethonium sensitive synaptic connections are first detected at 2-3 weeks in vitro. As these cells mature in culture the number of observed synaptic potentials increases, suprathreshold responses are more frequent, and a greater complexity of interconnections occurs between cells. These findings correlate closely in time with a shift seen in the synaptic vesicle population from dense core to clear (Johnson, et al., this volume) and increases in choline acetyltransferase activity (Ross and Bunge, this volume) seen in parallel cultures.

The finding that these cells change their sensitivity to guanethidine as they mature in vitro, coupled with the observation that cholinergic sympathetic neurons are resistant to the effects of the drug in vivo (Burnstock, et al., 1971) and the anatomical and biochemical data mentioned above, is consistent with the idea that there is a single cell type, normally adrenergic, which is induced to become cholinergic in our culture environment. Supported by USPHS Grant NS 05378 and NS 11888.

1097 EPINEPHRINE-STIMULATED CYCLIC AMP FORMATION IN SPECIFIC REGIONS OF MEDULLA OBLONGATA OF NORMAL RATS AND RATS WITH SPONTANEOUS HYPERTENSION (SHR). D. Wilkening*, B. Dvorkin* and M. H. Makman*. Albert Einstein Col. Med., Bronx, N. Y. Y. Matsumoto*, J. Y. Lew* and M. Goldstein, New York University Med. Ctr., New York, N. Y. and K. Fuxe*, Karolinska Inst., Stockholm, Sweden. (SPON: E. B. Gardner)

Neurons containing phenylethanolamine-N-methyl transferase (PNMT) and probably also epinephrine (Epi) have cell bodies which are localized in C1 and C2 cell groups in the medulla oblongata. We previously reported that at the level of the C1 region in the medulla oblongata Epi-stimulated adenylate cyclase activity (of homogenates) is selectively localized in the C1-surrounding region (containing Epi-nerve terminals) and also in C1 itself (containing both nerve terminals and cell bodies). Based on newer histofluorescence data we now have examined a portion of the C1 region which contains essentially only cell bodies. For these studies we have measured the stimulation by Epi of cyclic AMP (cAMP) formation in intact tissue incubated in vitro (Wilkening and Makman, Brain Res., 92, 572, 1975). Epi stimulated cAMP formation in C1 tissue containing only cell bodies as well as in C1-surrounding area containing nerve terminals. Stimulation by 10^{-4} M Epi was blocked 50-100% by $1\text{-}5 \times 10^{-5}$ M yohimbine. Both piperoxane and propranolol also antagonized the Epi-stimulation but were less potent than yohimbine. A possible interaction of yohimbine and piperoxane with hypothalamic receptors postsynaptic to Epi-neurons has been postulated previously (Bolme et al, Eur. J. Pharmacol., 28, 89, 1974). Certain of these Epi-containing neurons have cell bodies in the C2 region are involved in control of vascular tone and blood pressure (N. tr. solitarii and N. tr. vagi). An increased PNMT activity in both C2 and C1 regions of rats with spontaneous hypertension (SHR) was recently reported (Saavedra et al, Science 191, 483, 1976). We therefore studied the response to Epi of intact C2, C1 and C1-surrounding tissue from normal and SHR rats. Basal cAMP levels in all 3 regions were unchanged in SHR. However, the levels of cAMP after exposure for 5 minutes to 10^{-4} M Epi were significantly greater ($p < 0.01$) in C2 from SHR than from control rats, with an increment in cAMP due to Epi several times as great in SHR as in normal. Epi-stimulated cAMP levels in C1 and C1-surrounding area were not significantly increased in SHR over normal. Thus, selective enhancement of an Epi-sensitive adenylate cyclase occurs in the C2 region of hypertensive rats, implicating a role for this system in vasomotor function of C2 neurons.

1098 USE OF THE ENZYME PHENYLALANINE-AMMONIA-LYASE TO ENHANCE THE FLUORESCENT MICROSCOPIC STUDY OF SEROTONIN NEURONS. P. M. Adams, E. S. Barratt, R. R. Fritz* P. L. Poffenbarger* and C. W. Abell* Dept. Psychiatry, Human Biological Chemistry and Genetics, and Internal Medicine, Univ. of Texas Medical Branch, Galveston, Texas, 77550.

Previous research has shown that phenylalanine-ammonia-lyase (PAL), administered systemically, selectively reduced the plasma and brain levels of phenylalanine and tyrosine in monkeys and rats. This research also demonstrated an elevation in brain serotonin levels at 4 and 8 hours after a single PAL injection. The present study presents data which suggest that the study of serotonin neuronal pathways can be enhanced by the administration of PAL (100 units/kg, given IP) prior to sacrifice. Histofluorescent study of rat brain from animals sacrificed at 4, 8 or 24 hours following PAL injection showed enhanced detection of serotonin containing neurons. The enhancement was greatest at 8 and 24 hours after injection. These results suggest that the study of serotonin neural pathways can be greatly enhanced through the use of PAL and the careful selection of sacrifice time following the injection of the enzyme.

1099 RELEASE OF ENDOGENOUS NOREPINEPHRINE AND DOPAMINE IN VITRO. G. Jean Balcom and James L. Meyerhoff. Div of Neuropsychiatry, Dept Neuroendocrinology, Walter Reed Army Inst. of Research, Washington, D.C. 20012.

One criterion to be satisfied by putative neurotransmitters is that they be released during nerve stimulation. Because of the difficulties in measuring the small absolute amounts of transmitter released, most release studies have investigated the release of previously accumulated isotope. Recently very sensitive methods have become available for the measurement of picomole quantities of NE and DA. In this study we have examined some basic parameters of the release process for endogenous NE and DA. NE release was studied in an in vitro hypothalamus preparation. Chopped, washed tissue suspensions in Krebs-bicarbonate buffer were incubated for 10 minutes under various experimental conditions. The release process was stopped by centrifuging the incubation tubes at $4^{o}C$ for 10 minutes. Measurable quantities of NE were released into the medium spontaneously during a 10 minute incubation at $37^{o}C$. High potassium concentrations increased the NE outflow up to 200% of baseline levels. The KCl-evoked outflow was not due to inhibition of uptake of spontaneously released amine since KCl had no effect on uptake of ^{3}H-NE. The omission of Ca^{++} from the medium greatly diminished the KCl-stimulated release of NE, but did not affect spontaneous release of NE. The release of DA from chopped, washed striatal tissue was also investigated. In these experiments tissue was generally incubated for 2 minutes before release was terminated by separation of tissue and media on a millipore filter apparatus. KCl and amphetamine released DA in a concentration-dependent manner. Omission of Ca^{++} from the medium had no effect on the release of DA.

1100 EFFECTS OF OCTOPAMINE AND OTHER AMINES ON cAMP LEVELS IN LOBSTER TISSUES. B.-A. Battelle* and E.A. Kravitz (Spon. by E.A. Kravitz). Dept. Neurobiology, Harvard Med. Sch., Boston, MA 02115

Octopamine apparently acts as a neurosecretory substance in the lobster Homarus americanus. In the present study we compare the effect of octopamine and other amines on cAMP levels in three potential lobster target tissues; haemolymph, heart and exoskeletal muscle. Octopamine added to haemolymph causes a detectable increase in cAMP within 0.5 min. Threshold of the effect is at 10^{-7}M and maximum responses (up to 6-fold) are seen at 10^{-6}M DL-octopamine. Removal of haemocytes from haemolymph eliminates the increase. Dopamine and serotonin (10^{-4}M) and tyramine, histamine or norepinephrine (10^{-5}M) do not change cAMP levels in haemolymph. Thus a specific octopamine receptor seems to be present on haemocytes. The only apparent physiological effect of octopamine in haemolymph is an acceleration of haemolymph clotting. Octopamine and other amines cause increases in cAMP levels in exoskeletal muscle preparations and in heart. At the highest amine concentration tested on exoskeletal muscle (10^{-4}M), cAMP levels increase 2 to 3-fold with octopamine and dopamine and 14-fold with serotonin. The octopamine effect is specific for the D(-) isomer suggesting the existence of a specific receptor site on muscle. Further support for this idea comes from studies showing that LSD blocks the serotonin mediated increase in cAMP without affecting the octopamine increase. Attempted correlations of cAMP increases with physiological effects on muscle show no clear pattern. Octopamine and serotonin cause long lasting increases in contraction strength and muscle tension while dopamine causes the muscles to relax. LSD, which alone causes less than a 2-fold increase in cAMP elicits effects on contraction comparable to those produced by serotonin. In heart, where both octopamine and serotonin cause large increases in cAMP levels, octopamine does not and serotonin does increase the rate and strength of contraction. (Supported by NIH and the MDAA.)

1101 INFLUENCE OF NERVE SECTION, AXONAL FLOW AND ACTIVITY ON ACETYLCHOLINE SYNTHESIS IN NERVE TERMINALS. Robert L. Beach* and Guillermo Pilar. Biol. Sci. Group, Univ. of Conn., Storrs, Conn. 06268

In the chick iris - ciliary nerve preparation, acetylcholine (ACh) is synthesized from choline (Ch) accumulated via a high affinity transport system localized in the ganglion cell nerve terminals. Four hr after section of the ciliary nerves the high affinity Ch uptake and ACh synthesis are reduced, and by 72 hr no ACh is formed and all Ch transport is by a low affinity system. Section of the preganglionic oculomotor nerve does not decrease ACh synthesis of Ch uptake between 1 and 22 hr. Lidocaine applied locally to the ciliary nerves reversibly blocks conduction but does not affect Ch uptake or ACh synthesis relative to contralateral controls after 4 hr. Four or 7 hr after local application of colchicine (CLC) to the ciliary nerves, no decrease in Ch uptake or ACh synthesis is seen. CLC applied in this manner has been shown to mimic some aspects of axotomy (Pilar & Landmesser, Science 177: 116, 1972). Thus the decrease in Ch uptake and ACh synthesis 4 hr after nerve section is due to some factor other than block of activity or axonal flow.

During long term nerve block by lidocaine, or after recovery of nerve conduction, Ch uptake is reduced. Two weeks after application of CLC, Ch uptake is also reduced. Two to 3 days after section of the preganglionic nerve, ACh synthesis is reduced. Thus the long term maintenance of ACh synthesis in the nerve terminals may depend on some factors supplied by the neuronal somas. Different mechanisms are probably involved in the short term effects on transmitter synthesis after nerve section and long term effects seen after alteration of activity and axonal flow.

(Supported by NIH grant NS 10338 and the Univ. of Conn. Research Foundation).

1102 ANGIOTENSIN II AS A POSSIBLE MAMMALIAN CENTRAL NEUROTRANSMITTER:SYNAPTIC NEUROCHEMISTRY IN NORMAL MAMMALIAN AND HUNTINGTON CHOREA BRAIN TISSUE. James P. Bennett, Jr., Alberto Arregui*, and Solomon H. Snyder, Depts. of Pharmacology, Neurology, and Psychiatry, Johns Hopkins Med. Sch., Baltimore Md. 21205.

The octapeptide angiotensin II (AII) functions peripherally as a potent vasoconstrictor and stimulus for aldosterone secretion. Selective microinjection of AII into mammalian brain causes compulsive drinking behavior or increased sympathetic discharge resulting in increased heart rate and blood pressure. Angiotensin converting enzyme (ACE) is a specific dipeptidase which hydrolyzes the C-terminal His-Leu residue from the decapeptide AI yielding AII. ACE activity shows a 7-fold variation in 32 regions of calf brain with highest activity in the post. pituitary, globus pallidus, and pineal gland. ACE activity is significantly reduced to 20-40% of control values in caudate n., globus pallidus, and putamen tissue samples of patients dying from Huntington's Chorea, an autosomal dominant neurodegenerative disease affecting primarily the corpus striatum. With special incubation techniques that eliminate detectable peptide degradation, high affinity binding sites (K_d=0.2 nM) for ^{125}I-AII are demonstrable in rat and calf brain membranes. These binding sites show pronounced regional variations in rat and calf brain and possess peptide substrate specificities very similar to ^{125}I-AII binding sites in bovine adrenal cortex, a peripheral organ with receptors for AII. An active transport system for AII can be detected in synaptosomal fractions of brain tissue. This transport system possesses radically different kinetic properties compared to the AII binding process. Taken together, these neurochemical findings suggest a specialized role for AII in the brain, possibly as a neurotransmitter.

1103 DOPAMINE RECEPTOR BINDING: INFLUENCES OF AGE, CHRONIC DRUGS AND SPECIFIC LESIONS. David R. Burt, Ian Creese, José Pardo*, Joseph T. Coyle and Solomon H. Snyder. Dept. Pharmacol., Sch. Med., Johns Hopkins Univ., Baltimore, MD. 21205

[^{3}H]Haloperidol and [^{3}H]dopamine have previously been shown to bind to mammalian CNS dopamine receptors (Life Sci. 17:993, 1975). Binding of [^{3}H]haloperidol to striatal membranes of neonatal rats remains at a near constant value of less than 20% of adult levels between birth and 8 days of age. After this period, binding increases sharply, reaching adult levels by 45 days of age. In adult rats, chronic treatment with haloperidol (0.5 mg/kg/day for 3 weeks followed by 1 week drug-free) and other active neuroleptics increases binding of [^{3}H]haloperidol and [^{3}H]-dopamine to striatal membranes by about 25%. Higher doses of haloperidol do not appreciably increase binding above this level. Smaller increases are observed following shorter drug treatments. Greater increases in striatal binding (50%+) occur following specific lesions by 6-OH-dopamine of the substantia nigra. These increases in dopamine receptor binding appear to correlate to some degree with "behavioral supersensitivity." (Supported by USPHS grants MH-18501, DA-00266, MH-33128, DA-05328 and NS-01654.)

1104 CHARACTERIZATION OF β-ADRENERGIC RECEPTOR BINDING IN MAMMALIAN TISSUES. David B. Bylund, Michael E. Charness* and Solomon H. Snyder. Depts. of Pharmacol. and Psychiatry, Johns Hopkins Univ. Medical Sch., Baltimore, MD. 21205

Recently techniques have been developed in several laboratories for the biochemical labeling of neurotransmitter and hormonal receptor sites using high affinity radioactive agonists and antagonists. For the characterization of the β-adrenergic receptor, we have used two antagonists: ^{125}I-hydroxybenzylpindolol (HYP) and [^{3}H]-dihydroalprenolol. Both the in vivo and in vitro binding of these two ligands in mammalian tissues such as brain, heart and red blood cells display the properties expected for the labeling of the physiologically relevant receptor: rapid; reversible; saturable; and stereospecific. Numerous β-adrenergic antagonists and agonists inhibit the binding of the radioactive ligands at concentrations which are consistent with their known pharmacological potencies. In vitro drug specificities suggest that the β-receptors of rat brain are of the β_1 type while those on erythrocytes are of the β_2 type. The density of β-adrenergic binding sites in membrane preparation from rat erythrocytes is 150 fmol/mg protein; the K_D for ^{125}I-HYP binding is 25 pM. Using these membrane preparations, we have developed a simple and sensitive radioreceptor assay for propranolol and its active metabolites. In addition, we have compared the binding of ^{125}I-HYP with adenylate cyclase activities (basal, NaF and isoproterenol stimulated) in erythrocyte membranes from reticulocyte rich (90%) and normal (1% reticulocytes) blood. The results indicate that the functions of catecholamine recognition and consequent adenylate cyclase response can vary independently and suggest that the receptor and the cyclase may be autonomous molecular entities. (Supported by NIH grants MH-05036 and MH-18501.)

1105 COMPARISON OF THE EFFECTS OF TWO DRUGS WHICH BLOCK IMPULSE FLOW IN DA NEURONS ON STRIATAL ACETYLCHOLINE. R. Leslie Choi* and Robert H. Roth, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06510

The effects of γ-hydroxybutyrate (GHB) and 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) on regional brain acetylcholine (ACh) concentrations were studied. Although both drugs have general anesthetic properties they are unique in that they also inhibit impulse flow of the dopaminergic nigro-neostriatal and mesolimbic neurons. GHB, HA-966 or saline was administered i.v. to rats. After 15 min., the rats were killed by microwave irradiation directed to the head, and the brains were dissected. The ACh level in brain regions rich in dopaminergic nerve terminals was assayed by gas chromatography. GHB administration (50-400 mg/kg) resulted in dose-dependent increase of striatal ACh level. The elevation of ACh level was related to the observed degree of CNS depression. However, administration of an anesthetic dose of HA-966 (50 mg/kg) resulted in a significant decrease rather than an increase in striatal ACh level. ACh levels in olfactory tubercle and frontal cortex were significantly elevated. Since both drugs inhibit dopaminergic neuronal impulse flow, the results are consistent with the following hypothesis. GHB, by directly inhibiting cholinergic neurons, may cause an elevation of striatal ACh. If HA-966 lacks this direct inhibitory effect on striatal cholinergic neurons, its effects on striatal ACh are probably best explained by the ability of this drug to block impulse flow in dopaminergic neurons. The removal of the normal inhibitory influence of the dopamine system on ACh containing striatal interneurons should cause an increase in the activity of these cholinergic neurons and a decrease in striatal ACh. Experiments are in progress to test this hypothesis. (Supported in part by a grant from the USPHS, MH-14092.)

1106 REGULATION OF DOPAMINE BETA-HYDROXYLASE IN RAT ADRENAL GLANDS. Roland D. Ciaranello, G.Frederick Wooten, and Julius Axelrod. Dept. Psychiatry, Stanford Medical Center and Lab. Clin.Sci. NIMH, Bethesda,Maryland.

Rat Adrenal gland levels of dopamine beta-hydroxylase are subject to dual control. Activation of the splanchnic nerves to the adrenal medulla by reserpine induces the synthesis of dopamine beta-hydroxylase without altering the rate of enzyme degradation. In contrast, hypophysectomy causes a decline in steady state dopamine beta-hydroxylase levels by first accelerating the rate of degradation, then by slowing the rate of enzyme synthesis as well. Adrenocorticotrophic hormone administration partially reversed the effect of hypophysectomy on dopamine beta-hydroxylase degradation.

These findings suggest that the trans-synaptic factors controlling dopamine beta-hydroxylase induction act by a different mechanism (enzyme synthesis) than the hormonal controls regulating steady-state levels (enzyme degradation) Thus, active inhibition of enzyme degradation may be an important control in maintenance of steady state enzyme levels. Despite these mechanistic differences, both systems require a normally innervated cholinergic receptor to exert their effect. The enzyme response to either neural stimulation or ACTH administration is blocked by splanchnic denervation. Glucocorticoid stimulation of DBH, however, can occur after adrenal denervation, suggesting that ACTH acts on a receptor which requires splanchnic innervation, but glucocorticoids act distal to the receptor.

1107 IMMUNOCYTOCHEMICAL STUDIES OF DOPAMINE-β-HYDROXYLASE IN THE LOCUS COERULEUS AND HYPOTHALAMUS. Donald L. Cimarusti*, Kihachi Saito*, Robert Barber*, Paul E. Thomas*, and Eugene Roberts. Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010 and the Department of Biochemistry and Drug Metabolism, Hoffman-La Roche, Nutley, New Jersey 07110.

Dopamine-β-hydroxylase (DBH) was localized immunocytochemically in the locus coeruleus and hypothalamus of the rat employing a recently developed peroxidase: anti-peroxidase - Fab complex. Specific rabbit antiserum against DBH purified from bovine adrenal medulla (Rush et al., B.B.R.C. 57: 1301, 1974) was applied to tissues prepared and processed by a modification of previously published procedures (e.g., McLaughlin et al., J. Comp. Neurol., 164: 305, 1975). At the light microscopic level, specific staining for DBH was observed in the cytoplasm of the somata and processes of neurons in the locus coeruleus and subcoeruleus. Specific staining for DBH was not observed in the nuclei of these neurons. The hypothalamus showed dense staining for DBH in the fibers of the dorsal tegmental bundle. Some of these fibers appeared to terminate as dense punctate structures, 1-2 μm in diameter, in the vicinity of neuronal somata located in the interstitial nucleus of the stria terminalis. Similar punctate structures, along with fiber staining, was observed to be sparsely distributed in various hypothalamic nuclei. Preliminary electron microscopic studies suggest that DBH reaction product is related to some cisternae of the granular endoplasmic reticulum within the neuronal somata of the locus coeruleus. (Supported by grants NS-01115 and NS-12116).

1108 ORAL CHOLINE ADMINISTRATION TO PATIENTS WITH HUNTINGTON'S DISEASE. Edith L. Cohen, John H. Growdon*, and Richard J. Wurtman. M.I.T. Boston, MA. 02139.

Choline administration increases choline (Ch) and acetylcholine (ACh) levels in rat brain, apparently by increasing the net catalytic activity of choline acetyltransferase; but the extent to which this occurs in humans is largely untested. Even so, Ch is now being given to patients with brain diseases, such as Huntington's disease (HD) and tardive dyskinesia, to correct postulated deficiencies in brain ACh by increasing the availability of its precursor, Ch. In order to determine the distribution of Ch in body fluids after such treatment, we gave Ch orally, 8-20 g./day in 4 divided doses, to 9 patients with HD. Blood and cerebrospinal fluid (CSF) were collected before treatment with Ch, and Ch levels determined by a radioenzymatic method. These values were then contrasted with those obtained 1 hour after a dose of Ch during chronic therapy.

FLUID	CHOLINE DOSE (g. 4 times/day)	BEFORE TREATMENT (nmols/ml.)	AFTER TREATMENT (nmols/ml.)
serum	2	13.6 ± 1.7	25.8 ± 1.7 **
CSF	2-5	1.8 ± 0.1	3.1 ± 0.3 **

** $p < .01$, Student's t test

There was a dose-related increase in serum Ch that was linear over a range of 8-20 g./day. Ch in the CSF also increased in all instances, but reached a maximum elevation (at 8 g./day) beyond which further increases in Ch dose did not cause further rises. These data indicate that oral Ch consumption can increase Ch levels in human body fluids. Such an increase in Ch levels may then cause increased ACh synthesis in humans, as well as in rats.

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1109 INFLUENCE OF ADRENAL INNERVATION ON CATECHOLAMINE SYNTHESIS DURING ETHANOL WITHDRAWAL. L.B. Cohen*, E.M. Sellers*, E.A. Sellers* and K.V. Flattery* (SPON: J. Scott). Addiction Research Foundation, and Dept. of Pharmacology, University of Toronto, Toronto, Ontario, Canada.

Alterations in peripheral catecholamine turnover during ethanol withdrawal may be mediated centrally by increased CNS sympathetic discharge or may be produced by direct effects on release and/or biosynthesis at the nerve terminal. Bilateral denervation of rat adrenals was accomplished by surgical transection of the splanchnic nerve branches innervating right and left adrenals. Denervated and sham operated rats were pair fed on equicaloric ethanol or sucrose diets for 30 days. The average daily ethanol intake was 12 g/kg, resulting in average blood ethanol concentrations of 1500 mg/l. Rats were sacrificed 8 hours after ethanol feeding was discontinued. Changes in tyrosine hydroxylase (TH) activity between denervated-sucrose and sham-sucrose treatment groups is not significant (n.s.); hence the effects of denervation is n.s. However, TH activity in the sham-ethanol group is 30% greater than in the sham-sucrose group ($p < 0.05$), and 88% greater than in the denervated-ethanol group ($p < 0.05$). The difference in TH activity between denervated-ethanol and denervated-sucrose groups is n.s. Dopamine-β-hydroxylase and phenylethanolamine-N-methyl transferase activities, and norepinephrine and epinephrine levels were determined concurrently, and the resulting patterns of change indicate that TH activity is the rate-limiting step. These studies show that ethanol withdrawal is associated with increased adrenal catecholamine synthesis, and that this increase is dependent upon intact neural input to the gland.

(Supported by the Addiction Research Foundation and MRC-MT2701).

1110 GAMMA AMINOBUTYRIC ACID (GABA) AS A POSSIBLE TRANSMITTER AT A CRAYFISH INHIBITORY SYNAPSE. W. Craelius* and R.A. Fricke* (SPON: J. Wine). Dept. of Biological Sciences, Stanford University, Stanford, CA. 94305.

A study was undertaken to determine if GABA is a neurotransmitter at the accessory fiber synapse on the crayfish muscle receptor organ (MRO). Locally applied pulses of current from micropipets filled with a solution of GABA (pH 4.5) produce inhibition of impulses in crayfish MROs similar to that produced by stimulation of their accessory fibers. Small current magnitudes (0-10 namps) are sufficient to inhibit impulses when micropipets are positioned within the dendritic region of MROs possessing accessory innervation. The current magnitude required for inhibition on the soma and axon increases as a function of distance from the dendrites. Current applied to MROs in the sixth abdominal segment, which possesses no accessory innervation, does not inhibit impulses. Inhibition by GABA thus appears to be dependent upon the presence of inhibitory synapses and is most effective where the synapses are most numerous.

In the second part of this study, uptake and transport of GABA by accessory motoneurons was examined by incubating chains of two ganglia in ^{3}H-GABA (10^{-5} M). Because accessory motoneuron cell bodies lie one segment more caudal than the ganglion from which their axons exit, each ganglion pair contains both intact accessory axons as well as control axons disconnected from their somata. A time-dependent rise in radioactivity was found only in the saline bathing intact accessory motoneuronal terminals. Moreover, the rate of transport under a variety of experimental conditions consistently falls in the range of 250 to 350 mm/day.

Since GABA sensitivity is normally localized to the dendritic region of innervated MROs, it will be of interest to learn if degeneration of these synapses results in a spread of GABA sensitivity.

1111 GLYCINERGIC PATHWAY IN THE PIGEON OPTIC LOBE AND RETROGRADE MIGRATION OF MATERIAL TAKEN UP AS AMINO ACID. M. Cuénod, D. Felix*, H. Henke*, S. Hunt*, H. Künzle*, D. Le Fort*, J.C. Reubi*, T. Schenker* and P. Streit*. Brain Research Institute, Zurich University, CH-8029 Zurich.

The existence of nerve terminals using glycine as transmitter in the pigeon optic tectum is suggested by the following observations: (a) Glycine is taken up in synaptosomal fractions with high affinity (K_T: 3,5 . 10^{-5} M). (b) Specific strychnine-binding in the tectum membranes indicates the presence of glycine receptors. (c) Iontophoretic application of glycine depresses the activity of tectal units.

The N. isthmi, pars parvocellularis (Ipc) is proposed as one nucleus of origin for these glycine specific terminals since: (a) Ipc cell bodies send axons to superficial layers of the tectum, as demonstrated by autoradiographic and HRP anterograde tracing. (b) Electrical stimulation of Ipc induces a release of exogenous glycine in the tectum. (c) Ipc lesion induces a decrease in glycine high affinity uptake. (d) After ^{3}H-glycine application in the tectum, Ipc cell bodies are intensely labeled in a topographical manner; this can also be observed with ^{3}H-serine and ^{3}H-alanine, but not with many other amino acids. Serine and alanine are also taken up by high affinity systems which are competitively inhibited by glycine.

In summary, these results suggest that the Ipc-tectal pathway is glycinergic and that after transmitter labeling of the terminals, the radioactivity migrates toward the cell bodies.

1112 [^{3}H] DIHYDROERGOCRYPTINE (DHE) BINDING SITES IN RAT BRAIN. James N. Davis, Warren Strittmatter*, Elizabeth Hoyler*, and Robert J. Lefkowitz*. Duke University Medical Center, Durham, North Carolina 27705.

[^{3}H] DHE appears to bind to α-adrenergic receptors in rabbit uterine membranes. We have studied the binding of [^{3}H] DHE to membranes from rat cerebral cortex. The association and dissociation kinetics of binding were somewhat slower in brain than in uterine membranes although the affinity of binding (K_D = 8 nM) was similar. The potency of a large number of adrenergic agonists, antagonists and related agents in displacing [^{3}H] DHE from its binding sites was determined and differed significantly from results obtained in uterine membranes. The order of potency for agonists was: clonidine = (-) epinephrine (EPI) > (-) norepinephrine (NE) > (-) phenylephrine >> (-) isoproterenol, however approximately 10 fold higher concentrations of agents were required in brain than in uterus. [^{3}H] DHE binding showed stereospecificity; 5-10 fold higher concentrations of (+) EPI and (+) NE were required for displacement equivalent to the (-) isomers in the brain while in the uterus 20-30 fold stereospecificity was observed. Serotonin, apomorphine, and dopamine were also quite potent in brain displacing [^{3}H] DHE binding in brain membranes roughly equivalent to (-) EPI and (-) NE, whereas these agents were very weak competitors for [^{3}H] DHE binding in the uterus (< 1/100th as potent as (-) EPI). The order of potency for antagonists was: DHE > phentolamine >> chlorpromazine > phenoxybenzamine > (-) propranolol > haloperidol. The distribution of [^{3}H] DHE binding sites in rat brain was strikingly similar to β-adrenergic receptor binding sites with the exceptions of the hypothalamus and spinal cord (more [^{3}H] DHE binding sites) and the cerebellum and corpus striatum (more β-adrenergic receptor sites). The relevance of these DHE binding sites to known neurotransmitter receptors will be discussed.

1113 ANTAGONISM BY PICROTOXIN OF 5-HYDROXYTRYPTAMINE INDUCED DEPOLARIZATION AND EXCITATION OF THE NODOSE GANGLION OF THE CAT. William C. de Groat and William Simonds*. Dept. Pharmacology, School of Medicine, U. of Pittsburgh, Pittsburgh, PA 15261

Depolarization of the nodose ganglion and firing on the adjacent peripheral trunk of the vagus nerve in response to the close intra-arterial injection of 5-hydroxytryptamine (5HT) were reversibly antagonized by picrotoxin (PT). This result is consistent with earlier studies in which PT blocked the excitatory effects of 5HT on both sympathetic and parasympathetic ganglion cells.

Negative potentials ranging in amplitude to 900μV and in duration to 10 seconds were recorded from the surface of the nodose ganglion after injections of 5HT (0.02-100μg). 5HT also elicited a discharge in the vagus nerve, apnea and a transient fall in blood pressure. The responses were undiminished after section of the carotid sinus or glossopharyngeal nerves, however, the discharge was greatly reduced and the apneic response abolished after vagal section central to the nodose ganglion. All responses were reduced by the intra-arterial administration of PT (10-100 μg). The antagonism could be overcome by increasing the dose of 5HT. The effect of PT persisted for 10-20 minutes. The intra-arterial administration of GABA (0.2-200 μg) elicited a more prolonged (24-36 seconds) depolarization of the nodose ganglion, but did not produce vagal firing. The GABA-depolarization was also antagonized by PT (10-100 μg).

The interaction between PT and 5HT in the vagal ganglion raises the possibility that these agents might also have effects at vagal afferent terminals in the medulla. Thus, the current concepts regarding the mechanism of action of PT in the central nervous system might require modification to include effects at tryptaminergic as well as GABA-mediated synapses. Supported by NIH grants NS 07923 & NS 13854.

1114 RAPID ESTIMATION OF HOMOVANILLIC ACID FORMATION IN RAT BRAIN BY USE OF METHIONINE-CD_3. M. Ebert*, W. Havens*, K. Powers*, S. Markey*, and I. Kopin. NIMH, Bethesda, MD. 20014.

Measurement of the rate of formation of homovanillic acid-CD_3 (HVA-CD_3) from methionine-CD_3 (Me-CD_3) provides an estimate of the rate of dopamine (DA) metabolism. The levels and relative enrichments with CD_3 of Me and HVA in rat brain were determined by gas chromatography-mass spectroscopy after intravenous administration of Me-CD_3 (50 mg/kg). One minute after its administration, the relative enrichment of Me-CD_3 in brain was about 60% and declined exponentially with a half-life of about 70 minutes. The HVA-CD_3 content increased progressively and reached the level of Me-CD_3 enrichment in brain (35%) at about one hour. The rate of HVA formation was estimated 20 minutes after Me-CD_3 injection from the HVA-CD_3 content and relative enrichment of Me-CD_3. In otherwise untreated rats the rate of HVA formation was 144 $\pm$ 9.3 (SEM) ng/gm/hr. This rate of formation is consistent with estimates of rates of DA turnover (210 ng/gm/hr) obtained by other methods which require larger time intervals. One hour after administration of chlorpromazine (15 mg/kg) HVA formation was increased to 400 $\pm$ 17 µgm/ng/hr. Pretreatment with α bromoergocryptine (5 mg/kg), a DA agonist, produced a decrease in HVA synthesis at 3 hrs. to 62.7 $\pm$ 17.7 ng/gm/hr, a return to normal at 8 hrs., and a rebound increase in synthesis to 208 $\pm$ 15.6 ng/gm/hr at 16 hours. Apomorphine had similar effects, but a more rapid time course. The rapid turnover of HVA involves possible estimation of rates of DA metabolism over short intervals so that the time course of changes in DA metabolism can be examined.

1115 MEASUREMENT OF γ-AMINOBUTYRIC ACID IN HUMAN CEREBROSPINAL FLUID. S.J. Enna, J.H. Wood*, and S.H. Snyder. Dept. Pharmacol., Sch. Med., Johns Hopkins Univ., Balto., MD. 21205, and NINCDS, NIH, Bethesda, MD. 20014.

Lack of sufficient sensitivity and/or interfering substances have made the accurate determination of γ-aminobutyric acid (GABA) in cerebrospinal fluid (CSF) difficult. Recently we have reported a simple and specific radioreceptor assay for GABA in brain tissue with a sensitivity in microassay of about 10 pmoles (J. Neurochem. 26: 221, 1976). The principle of the assay is that ^{3}H-GABA bound to crude synaptic membranes, in the presence of added GABA, is inversely proportional to the logarithm of the added GABA. In the present study we report that preincubation of membranes in 0.05% Triton X-100 significantly increases the affinity of the membrane binding site for ^{3}H-GABA, improving the sensitivity of the GABA assay to about 1 pmole in a 100 µl assay. Unlike enzymatic-fluorometric assays, physiological salt concentrations do not interfere with the GABA binding process. When tritonized membrane fragments (0.5 mg protein) are incubated at 4° for 5-min in 2 ml of Tris-citrate buffer (pH 7.1) in the presence of 8 nM ^{3}H-GABA and 250 µl of human lumbar CSF, reduction of ^{3}H-GABA binding is equivalent to the presence of 225 $\pm$ 39 pmoles/ml GABA (n=11). Other qualitative tests indicate that the inhibiting substance behaves like authentic GABA. Lumbar CSF obtained from seizure patients or probenecid treated individuals yield values of 159 $\pm$ 17 (n=12) and 166 $\pm$ 25 (n=25) pmoles/ml respectively which are not significantly different from controls. GABA concentration increases progressively at higher levels of the CSF system suggesting that CSF GABA may reflect GABAergic activity in the brain. (Supported by USPHS grants MH-01598 (SJE), MH-18501 and RSDA MH-33128 (SHS)).

1116 AN ^{125}I-LABELED PROBE FOR CHOLINERGIC MUSCARINIC RECEPTOR. Steven D. Flanagan* and Angelo Storni* (SPON: A. DRAVID). Friedrich Miescher-Institut, Postfach 273, Basel, Switzerland and Pharma-Forschung, Ciba-Geigy Limited, Basel, Switzerland.

Sensitive in vitro assay of postsynaptic receptors is a necessary first step for their isolation and characterization. Assay of the muscarinic receptor has recently been accomplished using [^{3}H]-quinuclidinyl benzilate (QNB) [Yamamura, H.I. and S.H. Snyder, Proc. Nat. Sci. USA 71: 1725-1729, (1974)]. We have synthesized a QNB derivative, 3-quinuclidinyl-p-hydroxybenzilate (OH-QNB), which can be iodinated with ^{125}I, yielding a reagent of higher specific radioactivity. Specific binding of ^{125}I-OH-QNB, as measured by a filter binding assay (see above ref.), was saturable and reversible. The ratios of binding among rat brain mitochondria, synaptic plasma membrane and myelin fractions were similar for both [^{3}H]-QNB and ^{125}I-OH-QNB. The highest specific binding activity per mg protein was found in the synaptic plasma membrane fraction, where the half-life of the ^{125}I-OH-QNB-receptor complex was 1 to 2 hr at room temperature and 10 min at 35 degrees.

The relatively long half-life of the complex makes this a potentially useful reagent for assessing the levels of muscarinic receptor in samples containing only small amounts of binding sites.

1117 PRESENCE OF ADENYLATE CYCLASE SENSITIVE TO DOPAMINE AND NEUROLEPTIC DRUGS IN BRAIN REGIONS CONTAINING DOPAMINE-CELL BODIES: A9 (SUBSTANTIA NIGRA) AND A10. Eliot L. Gardner, B. Dvorkin*, Ram K. Mishra*, Robert Katzman and Maynard H. Makman*. Albert Einstein Col. Med., Bronx, N.Y. 10461.

The presence of dopamine (DA)-stimulated adenylate cyclase activity (AC) in regions of the central nervous system containing post-synaptic receptors for DA and also the post-synaptic localization of all or most of this AC have now been fairly well established. We now report the presence of DA-stimulated AC in brain regions containing major groups of cell bodies of DA-neurons, A9 and A10 in rat. A separate group of rats were used to confirm by histofluorescence techniques the accuracy and reproducibility of dissections. AC of tissue homogenates of both A9 and A10 was stimulated 2-3 fold by 10 μM DA with only a small and variable degree of stimulation by isoproterenol observed. Specific activity of rat A9 homogenates in the presence of DA was about 1/3 that of caudate nucleus homogenates; the % stimulation by DA, however, equals or exceeds that of rat caudate nucleus, olfactory tubercule or nucleus accumbens. AC of A9 was also stimulated by apomorphine and stimulation by 100 μM was blocked completely by 10 μM fluphenazine, but not at all by 10 μM propranolol. Stimulation by 10 μM DA was blocked effectively by 0.1 μM fluphenazine, 1 μM clozapine and 1 μM, but not 0.1 μM haloperidol. Pimozide was also less potent than fluphenazine as a blocking agent. In preliminary studies, after specific lesions of rat A9 by 6-hydroxydopamine, the DA-stimulated AC in A9 persists, suggesting that this activity may not be located on DA-cell bodies of the pars compacta but rather may be at a site post-synaptic to these cells within A9, possibly in the pars reticulata. The finding of DA-stimulated AC within A9 and A10 sensitive to neuroleptic drugs provides an additional site for influence of these drugs on extrapyramidal and mesolimbic systems.

1118 ADRENOCORTICAL STEROIDS ON PHENYLETHANOLAMINE N-METHYLTRANSFERASE ACTIVITY AND EPINEPHRINE CONTENT OF SYMPATHETIC GANGLIA OF NEONATAL RAT. G. Gianutsos and K.E. Moore. Dept. of Pharmacology, Michigan State University, East Lansing, Michigan, 48824.

Administration of dexamethasone (DEX) to neonatal rats increases the activity of phenylethanolamine N-methyltransferase (PNMT; Ciaranello et al., J. Neurochem. 20: 799, 1973) and the content of epinephrine (E; Koslow et al., J. Neurochem. 24:277, 1975; Moore and Phillipson, J. Neurochem. 25:289, 1975) in the superior cervical ganglion (SCG).

Injections of DEX (0.1 mg/kg/day, s.c.) on each of the first three days of life increased the PNMT activity and E content without altering the dopamine and slightly reducing the norepinephrine contents of the SCG and the stellate ganglion. The PNMT activity and E content in SCG remained elevated for 10-14 days. DEX did not alter PNMT activity or catecholamine content of salivary glands or the heart. Administration of DEX (2 mg/kg, s.c.) to pregnant rats on days 19, 20 and 21 of gestation increased the PNMT activity and E content in SCG of newborn rats. PNMT activity in SCG of neonatal rats was also increased by injections of ACTH (25 mu bid/rat for 5 injections) or reserpine (1 mg/kg), both of which increase the plasma concentration of corticosterone in adult rats. Exposure to a cold stress (60 min at 4°C qid for 3 days) also increased PNMT activity in SCG. These results indicate that the actions of DEX on neonatal sympathetic ganglia are reproduced by increasing plasma concentrations of endogenous adrenocortical steroids. (Supported by a grant from the Michigan Heart Association.)

1119 DIFFERENTIAL LABELING OF AGONIST AND ANTAGONIST STATES OF CENTRAL α-NORADRENERGIC RECEPTORS. David A. Greenberg*, David C. U'Prichard* and Solomon H. Snyder. Depts. Pharmacology and Psychiatry, Johns Hopkins Medical School, Baltimore, Md. 21205.

The α-noradrenergic agonist, [^{3}H]clonidine, and the α-noradrenergic antagonist, [^{3}H]WB-4101, show high affinity, saturable binding to mammalian brain membranes, with K_D's of 5 nM and 0.6 nM respectively. The ligand-displacing affinities of phenylethylamines correlate with their pharmacologic α-agonist potencies, with epinephrine > norepinephrine >> dopamine > isoproterenol. Binding of both ligands is stereoselective, (-)-catecholamines being 10-50 times more potent than the corresponding (+)-isomers. α-Agonists are much more potent displacers of [^{3}H]clonidine than of [^{3}H]WB-4101, while α-antagonists displace [^{3}H]WB-4101 more readily. Ergot alkaloids, which are mixed agonist-antagonists, have similar displacement affinities for the two ligands, and only those ergots known to be pharmacologically active at the α-receptor are potent ligand displacers. Destruction of central catecholamine nerve terminals by 6-OHDA does not reduce the binding of either ligand, suggesting that binding occurs at post-synaptic sites. Binding of the two ligands shows a similar regional distribution within the C.N.S., with the hypothalamus and cerebral cortex displaying highest binding and the cerebellum least. Various ionic treatments differentially affect the binding of agonists and antagonists. These findings suggest that [^{3}H]clonidine and [^{3}H]WB-4101 preferentially bind to respective agonist and antagonist states of the post-synaptic α-noradrenergic receptor. (Supported by USPHS grants MH-18501, MH-33128 and MH-05105.)

1120 NOREPINEPHRINE UPTAKE FOLLOWING ACUTE HEAD TRAUMA IN RATS USING A KINETIC PLOT ADAPTED FOR ANALYSIS OF WHOLE BRAIN SYNAPTOSOMES. M. Gary Hadfield* William E. Adams, Donald P. Becker*, and William F.C. Rigby. Divisions of Neuropathology and Neurosurgery, Med. Coll. Va., Richmond, Va. 23298

Adult male Sprague-Dawley rats received acute head trauma in a free fall accelerating--decelerating device which delivered a force of 1.31 Kg. meters. Controls were allowed to fall but without impact. Whole brain crude synaptosomes were obtained and were incubated with tritiated l-norepinephrine (^{3}HNE). The traumatized animals showed an increase in both maximum uptake velocity and Km (decreased affinity) for ^{3}HNE. These plastic changes in transport membranes may be related to the production of coma following head trauma since reuptake is a principal mechanism for inactivation of neurotransmitter.

However, we were unable to plot Km and Vmax using conventional double reciprocal and V vs. V/S plots. These produced curved instead of straight lines since uptake was greater at the higher concentrations. This effect is presumed due to the presence of myelin, diverse populations of synaptosomes, and/or multiple uptake mechanisms. Squaring of the substrate concentration again produced straight lines which could be easily analyzed by Michaelis-Menten kinetics (V vs. V/S^2) thus making feasible kinetic analysis of neurotransmitter uptake in whole brain crude synaptosome fractions.

1121 ONTOGENY OF β-ADRENERGIC RECEPTORS AND CATECHOLAMINE RESPONSIVENESS IN RAT CEREBRAL CORTEX. T.K. Harden, B.B. Wolfe,* J.R. Sporn,* J.P. Perkins,* and P.B. Molinoff. University of Colorado Medical Center, Denver, Colo.

A high affinity β-adrenergic receptor antagonist, [^{125}I] iodohydroxybenzylpindolol (IHYP; Aurbach, et al., Science, 1974) has been used as a ligand in a binding assay to study the development of β-adrenergic receptors in rat cerebral cortex. Specific binding of IHYP was barely detectable during the first week after birth. Between days seven and fourteen there was a rapid increase in the density of receptors. Adult levels (0.3-0.4 pmol/mg) were reached by the end of the second week after birth. The affinities of l-isoproterenol (0.3μM) and IHYP (1.5nM) for β-adrenergic receptors did not change with the age of the animal. The ontogeny of catecholamine responsiveness in the cerebral cortex followed a similar time course to that of β-adrenergic receptors. Isoproterenol stimulated cyclic AMP accumulation was negligible during the first week after birth, but it increased rapidly to adult levels between days seven and fourteen. On the other hand, fluoride stimulated adenylate cyclase activity in the cerebral cortex was 0.21 ± .02 nmol/mg/min at birth and gradually increased to maximal activity (0.64 ± .04 nmol/mg/min) over the next three weeks. Norepinephrine stores and dopamine-β-hydroxylase activity in the cerebral cortex developed with a slow time course reaching adult levels approximately two months after birth. Thus, there was no correlation between the time course of development of markers for presynaptic adrenergic nerve terminals and the postsynaptic ontogeny of β-adrenergic receptors. The results suggest that in the rat cerebral cortex, the catecholamine receptor-adenylate cyclase system does not develop as an intact functional unit, but rather, catalytic sites and receptor molecules appear to develop independently. It is likely that the development of β-adrenergic receptors permits the expression of catecholamine sensitive adenylate cyclase.

1122 NEUROTRANSMITTER RELEASE FROM BRAIN: I. COOPERATIVE OR NON-COOPERATIVE INVOLVEMENT OF CALCIUM? John W. Haycock, William B. Levy and Carl W. Cotman. Dept. Psychobiology, UC Irvine, CA 92717 and Dept. of Psychology, UC Riverside, CA 92502.

The release of accumulated neurotransmitters from rat brain synaptosomal fractions was investigated as a function of $[Ca^{++}]_o$. In other well-studied stimulus-secretion coupling systems, the relationship between $[Ca^{++}]_o$ and neurotransmitter secretion has been employed for inferring the mechanism by which calcium triggers the secretion process. Following the suggestion of del Castillo and Katz (1954) and Jenkinson (1957), Dodge and Rahamimoff (1967) invoked the reaction

$$nCa + X \rightleftharpoons Ca_nX$$

wherein n molecules of calcium react simultaneously with a release site (X) to produce a secretion event. Within this model, the maximum slope of the log-log relation between release and $[Ca^{++}]_o$ would reveal the parameter n.

Calcium-dependent release of accumulated neurotransmitters in the presence of agents that facilitate calcium influx was evaluated at low $[Ca^{++}]_o$ (0.05-0.5 mM) in terms of the log-log relation. For a variety of conditions (neurotransmitter, brain area, facilitating agent), the maximum slope of this relation was slightly less than 2. A similar slope for evoked release at room temperature is also observed at the mammalian neuromuscular junction (e.g., Oberg & Kelly, 1976).

Within the preceding model, the present data implicate a 2nd order reaction in which 2 molecules of calcium must combine with a postulated release site in order to produce a release event. An alternative model, in which the size of the readily releasable pool and the release process are *first* order reactions with calcium, is also discussed. The latter model of two sequential 1st order reactions allows for log-log slopes that can approach a value of 2. (Supported by NS 08597 and BMS 180809.)

1123 CORRELATION OF UNIT RESPONSES TO NARCOTIC DRUGS AND TO SOME PUTATIVE TRANSMITTERS IN CAT BRAIN STEM. James L. Henry, Dept. Anaesthesia Research, McGill Univ., Montreal, Canada.

A project was done to investigate a possible relation between the actions of narcotic drugs and of some putative transmitters when applied by microiontophoresis to single units in the brain stem of decerebrated, cerebellectomised cats. Drugs studied were: morphine (75 mM, pH 4.8), meperidine (100 mM, pH 5.2), Fentanyl (50 mM, pH 5.2), glutamate (1.0 M, pH 7.2), acetylcholine (ACh, 1.0 M, pH 4.2), norepinephrine (NE, 100 mM in 0.1% ascorbic acid, pH 3.9) and 5-hydroxytryptamine (5-HT, 100 mM, pH 4.1). A total of 66 units were studied; in the reticular formation (RF) 12 units were depressed, 7 were excited and 33 were unaffected by narcotic drugs; in the periaqueductal grey (PAG) narcotic drugs were without effect on 14 units studied. In the RF, ACh caused excitation of 18 and depression of 2 out of 34 units; , NE caused excitation of 12 and depression of 14 out of 37 units; 5-HT caused excitation of 20 and depression of 9 out of 47 units. In the PAG, ACh caused excitation of one and depression of 4 out of 7 units; NE caused excitation of 7 and depression of one out of 10 units; 5-HT caused excitation of 2 and depression of one out of 3 units. Correlation of responses to narcotic drugs with those to the putative transmitters showed that units which were excited by narcotic drugs were consistently excited by ACh and by 5-HT; NE had variable effects. Units depressed by narcotic drugs had inconsistent responses to ACh, NE and 5-HT. These results suggest that excitation of brain stem reticular units by narcotic drugs is related to excitation by ACh and 5-HT. (Supported by Cdn. MRC)

1124 GLYCINE AND GABA CONDUCTANCES IN LAMPREY INTERNEURONS. S. Homma* and C. Rovainen. Department Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110.

Two separate microelectrodes for passing current and for recording were placed in single giant interneurons in isolated spinal cords of adult lampreys while amino acids at 0.05-3 mM were applied in the bathing fluid at 6-9°.

Cellular conductance increased steeply with glycine concentration (cooperativity ≥ 2) to maxima 20-50 X that at rest. The conductance produced by glycine decreased approx. linearly with lowered external Cl and disappeared after washing in 0 Cl+glycine. Strychnine (10 μM) produced seizures in isolated lamprey spinal cord and was a competitive inhibitor of glycine. Bicuculline had no effect on glycine conductance.

Conductance produced by GABA was also Cl dependent and increased steeply with concentration. Bicuculline (2-10 μM) was a competitive inhibitor of GABA; strychnine had no effect. When responses to GABA desensitized, conductances could still be produced by glycine. Glycine was more effective in depleting internal Cl than was GABA. Uptake mechanisms for glycine and for GABA lowered the apparent sensitivity of the spinal cord to these compounds at 20°.

1125 EFFECTS OF PITUITARY-ADRENAL HORMONES ON CATECHOLAMINE SYNTHESIS IN MOUSE BRAIN. P.M. Iuvone*, R.L. Delanoy* and A.J. Dunn. Dept. Neuroscience, University of Florida, Gainesville, FL 32610.

Experiments with both humans and rodents have suggested that pituitary-adrenal hormones may play a role in physiological processes related to the acquisition and retrieval of certain types of information. Catecholamine neurotransmission and protein metabolism have also been implicated in these processes. Therefore, we examined the effects of several natural and synthetic analogs of pituitary-adrenal hormones on the incorporation of radioactivity from s.c. injected [^{3}H]tyrosine (TYR) into norepinephrine (NE), dopamine (DA) and protein in the brains of CD-1 mice. A single injection of corticosterone (5 or 15 μg/g) increased the conversion of TYR to NE in a dose-dependent manner. In three independent experiments, the daily injection of $ACTH_{1-24}$ (1 μg/g) was found to increase (nonsignificantly) the conversion of TYR to NE when measured about 24 hrs. after the last injection. Injections of $ACTH_{4-10}$, a synthetic analog with extremely low steroidogenic activity, resulted in significant increases in TYR conversion to both DA and NE. The effect of $ACTH_{4-10}$ was greater than that elicited by $ACTH_{1-24}$. The stimulation of catecholamine metabolism by $ACTH_{4-10}$ suggests that ACTH-like peptides (e.g. α- and β-MSH) may act directly on the CNS. However, adrenalectomy inhibited the $ACTH_{4-10}$-induced increase in NE and DA synthesis. Possible explanations of this phenomenon will be discussed.

In the same experiments the administration of $ACTH_{1-24}$, $ACTH_{4-10}$ or corticosterone had no effect on the incorporation of [^{3}H]tyrosine into brain proteins of intact or adrenalectomized CD-1 mice. This contrasts with the effects of ACTH on the [^{3}H]lysine incorporation into brain proteins of C57Bl/6J mice.

ACTH peptides were provided by Dr. H. Van Riezen of Organon. Supported by a grant from the U.S. National Institute of Mental Health (MH25486).

1126 TRANS-SYNAPTIC MODULATION OF DOPAMINE (DA) METABOLISM IN THE RAT SUPERIOR CERVICAL GANGLION. F. Karoum, C. K. Garrison,* N. H. Neff and R. J. Wyatt, Laboratory of Clinical Psychopharmacology and Laboratory of Preclinical Pharmacology, SMR, IRP, NIMH, St. Eliz. Hosp., Wash., D.C. 20032.

The influence of cholinergic preganglionic neurons on the metabolism of DA was measured from the changes in the concentrations of homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the ganglion of normal, bilaterally and unilaterally decentralized rats. HVA and DOPAC were assayed by mass fragmentography. DOPAC was the major metabolite of DA in the ganglion. Its concentration (41±3 pmol/ganglion) was about 20 times greater than that of HVA (2.7±0.5 pmol/ganglion). Probenecid (200 mg/kg ip) significantly increased the concentration of both metabolites suggesting that a probenecid-sensitive acid transport is, in part, responsible for their removal. Bilateral decentralization significantly reduced DOPAC while unilateral decentralization resulted in a fall in the decentralized ganglion, and a rise in the intact ganglion. Decentralization had no apparent effect on DA. Kinetic evaluation of the decline of DOPAC after pargyline (75 mg/kg ip) indicated that the rates of its formation in ganglia from control animals were 5.3, 4.0 and 7.8 pmol/ganglion/min, respectively. The rate constant for the decline of DOPAC was unaltered by the surgical procedures. We concluded that the rate constant for the efflux of DOPAC from the ganglia is independent of preganglionic input and that the concentration of DOPAC in the ganglion is a measure of its rate of formation. The fall and rise respectively in the rate of formation of DOPAC in the decentralized and intact ganglia suggests that a neuronal feedback loop influences the metabolism of DA. Thus, a reduced output of decentralized ganglion accentuates DA metabolism in the intact ganglion by feedback control.

1127 THE FLUX OF RADIOACTIVE LABEL THROUGH NINE COMPONENTS OF THE SEROTONERGIC SYSTEM. J.D. Lane* and M.H. Aprison. Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN. 46202.

Since the compartmentation of 5-hydroxytryptamine (serotonin) in nervous tissue is not firmly established, some insight into the question of multiple pools may be gained by studying the product-precursor relationships which exist between the major metabolites of the serotonergic system (principally tryptophan, 5-hydroxytryptophan, serotonin and 5-hydroxyindoleacetic acid) and the minor components which may contribute significantly (tryptamine, 5-methoxytryptamine, 5-hydroxyindolepyruvic acid, 5-hydroxytryptophol and protein synthesis utilizing tryptophan). The technical problems of separating and quantitating these components were solved with (a) use of a multiple assay (Smith et al., Anal. Biochem. 64, 149, 1975) and (b) paper chromatographic procedures. Rats were intravenously injected with a pulse of radioactively labelled L-tryptophan, were killed at various times post-injection and the brains were dissected into parts for analysis. The incorporation of label into and the flux of label through the major and minor components of the serotonergic system were measured in terms of specific activity (dpm/nmole) versus time. The minor components were detectable in some instances, but their overall radioactive contributions were slight, which ruled out possible metabolic shunts. Classical plots of specific activity versus time dictate that in a one compartment system the declining curve for a precursor must intersect its immediate product at the peak specific activity of the product. Anomolies were observed in the product-precursor relationships for 5-hydroxytryptophan and serotonin, as well as for serotonin and 5-hydroxyindoleacetic acid. These data could be explained by a mechanism involving the presence of multiple pools of serotonin. (Supported by Grant MH-03225-17 from NIMH).

1128 REGIONAL LEVELS OF NOREPINEPHRINE AND DOPAMINE IN RAT BRAIN AFTER MICROWAVE FIXATION AT 2450 OR 985 MHZ: POSSIBLE DIFFUSION ARTIFACT.
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High power microwave irradiation at 2450 MHZ has been used in the measurement of a number of metabolites (Cyclic AMP, Cyclic GMP, ATP, Creatine-P, etc.) and putative neurotransmitters (GABA, Glutamate, and Acetylcholine) in the brains of both rats and mice. Whole brain levels of these compounds have compared favorably to rapid freezing techniques. Although determination of many of these substances in brain regions has been performed after microwave inactivation, the possibility of cell membrane disruption secondary to rapid heating with subsequent diffusion has been considered but never demonstrated. Studies in our laboratory and one other have demonstrated the use of microwave irradiation at 2450 MHZ in the measurement of norepinephrine (NE) and dopamine (DA) in whole brain. Regional brain studies in our laboratory, however, indicate highly significant increases in DA concentration in specific areas including regions of the cortex, amygdala and septal area following irradiation at 2450 MHZ. Concentrations of NE in all 17 regions studied were comparable to the decapitated control values. Since the precise pattern of microwave energy deposition into the brain is not homogeneous and is affected by frequency as well as applicator characteristics, studies of regional levels of NE and DA in rat brain were carried out at 985 MHZ. Once again levels of DA were significantly elevated over decapitated controls in the same regions, while NE values remained unaffected. These data suggest that a problem of diffusion with microwave inactivation may occur independent of the frequency used, and may be significant in the case of compounds with high regional concentration gradients in brain.

1129 NEUROTRANSMITTER RELEASE FROM BRAIN: II. KINETIC PARAMETERS.
William B. Levy, John W. Haycock and Carl W. Cotman. Dept. Psychology, UC Riverside, CA 92502 and Dept. Psychobiology, UC Irvine, CA 92717.

The release of accumulated neurotransmitters from rat brain synaptosomal fractions was investigated as a function of $[Ca^{++}]_o$. Previously loaded tissue was exposed to intermediate $[Ca^{++}]_o$ (0.3-2.0 mM) for 20 sec in the presence of different agents that facilitate calcium influx. Maximum release (V_{max}) and the $[Ca^{++}]_o$ producing release equal to one half maximal release (K_m) were derived from linearizing plots. The apparent K_ms were similar across brain regions, neurotransmitters and levels of $[K^+]_o$. Ca-dependent release in the presence of veratridine required higher $[Ca^{++}]_o$ to produce half-maximal release.

The kinetic analysis was also applied to release from tissue previously stimulated with calcium (1.5 mM). Prior stimulation of release resulted in a selective decrease in maximal release without an apparent change in $[Ca^{++}]_o$ for half-maximal activation of the processes.

The similarity of K_ms for $[Ca^{++}]_o$ among the various neurotransmitters (GABA, NE, DA, ACh) and brain regions (cerebral cortex, corpus striatum) suggests a commonality for the mechanisms of calcium influx and/or calcium activation of stimulus-secretion coupling processes for different transmitter systems in brain. Differences in V_{max} of percent release may reflect differences in the relative partitioning of accumulated transmitter into readily releasable and storage pools. (Supported by Research Grants BMS 18089 to WBL and NS 08597 to CWC.)

1130 GABA BINDING IN HUMAN BRAIN: SPECIFIC ALTERATIONS IN SUBSTANTIA NIGRA OF PARKINSONIAN PATIENTS. K.G. Lloyd, L. Shemin* and O. Hornykiewicz. Clarke Institute of Psychiatry, 250 College St., Toronto, Canada, M5T 1R8.

Sodium-independent ^{3}H-GABA binding to crude membrane fractions from different areas of human brain (frozen postmortem material) was estimated by a modification of the method of Enna and Snyder (Br.Res. 100,81, 1975). The affinity constant(Kd) for GABA binding was similar in the human cerebellar cortex ($3.35 \pm 0.39 \times 10^{-7}$M) and whole rat brain ($5.10 \pm 0.40 \times 10^{-7}$M). The regional distribution of GABA binding in the human brain (no neurological disease) was, in decreasing rank order (values in brackets indicate relative binding at 25 nM ^{3}H-GABA): Cerebellar cortex (cbll cx, 100); Hippocampus (hippoc, 63); temporal cortex (57); amygdala (23); putamen (put, 21); caudate nucleus (CN, 20); substantia nigra (SN, 9); internal pallidum (5); substantia alba (0.5). The findings from patients with Parkinson's disease (5-9 cases) or Huntington's chorea (1-3 cases) are the following:

GABA Binding (% control)	Put	CN	SN	Cbll Cx	Hippoc	Pariet. Cx
Parkinson's disease	124	207	23*	124	87	175
Huntington's chorea	33	49	-	123	84	105

* $p<0.05$ as compared to controls

The only ^{3}H-GABA binding found to differ significantly from control was in the SN of patients with Parkinson's disease. Too few Huntington's patients were available for statistical analysis. This localized decrease in GABA binding in the substantia nigra from patients with Parkinson's disease may be taken as evidence for the existence of GABA receptors on dopamine cell bodies in the SN (which degenerate in Parkinson's disease).

Supported by the Clarke Institute of Psychiatry.

1131 TRANSPORT OF GAMMA-AMINOBUTYRIC ACID BY GLIAL CELLS IN CULTURE. D.Martin*, D.A.Brown* and W.Shain (SPON: D.L.Robinson)Neurobiology Dept., Armed Forces Radiobiology Research Institute, Bethesda,MD 20014.

GABA transport was studied in cultured glial cells obtained by dissociation of fetal or neonatal rat superior cervical ganglia and in a morphologically similar rat glioma cell line obtained from a spinal tumor(1056A cells) in order to confirm observations on glial transport previously made with intact superior cervical ganglia. The rate of uptake in HEPES buffered Hank's balanced salt solution was nearly constant for over two hours at 37° for all GABA concentrations studied. Kinetic analysis of uptake in 1056A cells revealed two components with apparent Michaelis constants of approximately 0.1 µM and 150 µM and a third component with an apparent K_m greater than 600 µM. Uptake of 0.025 µM GABA by 1056A cells was about 2 percent of control when NaCl was replaced with either choline chloride or sucrose whereas uptake of 10 µM and 1 mM GABA was not strongly sodium dependent. Ganglion glia also possessed a high affinity uptake system ($K_m \simeq 0.1$ µM). Uptake of 0.025 µM GABA by both ganglion glia and 1056A cells was much more strongly inhibited by β-alanine than by L-2,4-diaminobutyric acid. Representative α-amino acids (alanine, glycine, histidine, and leucine) were found to be extremely poor inhibitors of this uptake. The inhibitory potency of β-alanine relative to diaminobutyric acid was increased at 150 µM GABA. At this GABA concentration, the IC50 for β-alanine was less than 75 µM but 1.5 mM diaminobutyric acid inhibited by only 22 percent. The present results on substrate specificity, and Na dependence of the high affinity uptake system are similar to those obtained previously with intact superior cervical ganglia.

1132 MAPPING OF GLUTAMIC ACID DECARBOXYLASE (GAD) IN DISCRETE AREAS OF THE RAT BRAIN. II. HINDBRAIN (MESENCEPHALON, RHOMBENCEPHALON AND CEREBELLUM).
V. John Massari*, Zehava Gottesfeld* and David M. Jacobowitz, Lab. Clin. Sci., NIMH, Bethesda, MD. 20014.

GAD is the enzyme responsible for the biosynthesis of γ-amino butyric acid (GABA), a likely CNS neurotransmitter. The distribution in activity of this enzyme has been measured in order to map the location of neurons utilizing GABA for synaptic transmission. This was done by combining a sensitive radiometric assay for GAD with a "micropunch" method for removing individual nuclei from frozen brain slices. GAD was detected in all 40 nuclear areas examined. However, it showed a marked regional variation of distribution. GAD activity in the mesencephalon was highest in the substantia nigra reticulata (SNR) (595 ± 21 μmoles/ g protein/h), followed by substantia nigra compacta, superior and inferior colliculi, nucleus pretectalis, and several nuclei of the central gray. In the medullary reticular formation, most GAD values were low, averaging 15% of that found in the SNR. The cranial nerve nuclei showed somewhat higher values than this. A prominent exception to this pattern of results was found in the dorsal tegmental nucleus of Gudden (DTN). GAD values here were 60% of those in the SNR. This is noteworthy in light of known reciprocal connections of the DTN with several limbic and hypothalamic nuclei also rich in GAD. In the cerebellum, the dentate nucleus had 50% of the GAD activity found in the SNR, while the nucleus interpositus and nucleus fastigii showed somewhat less. These results suggest several promising avenues whereby surgical intervention of known neuroanatomical pathways may provide more information on the localization of GABAergic neuronal pathways in the CNS. Such studies are presently under way.

1133 INCOMPLETE SATURATION OF L-GLUTAMATE DECARBOXYLASE (GAD) WITH PYRIDOXAL-5'-PHOSPHATE (PLP) IN VIVO.
L.P. MILLER*, D.L. MARTIN* AND J.R. WALTERS. Lab. of Neuropharmacology, NINCDS, Bethesda, MD. 20014 and Dept. of Chemistry, University of Maryland, College Park, 20742.

The degree of saturation of GAD (EC 4.1.1.15) by PLP was determined in a high speed supernatant of 10% rat brain homogenate which had been subjected to chromatography on Sephadex G-25 to remove endogenous PLP and other possible GAD effectors. Activity measured in the absence of PLP was 83% of the activity observed in the presence of a saturating concentration of PLP. The following experiments were performed to determine whether this value represents the degree of saturation of the enzyme in vivo. When brains were collected by freeze-blowing to minimize postmortem changes, the level of saturation fell to 66%. Analysis showed the concentration of PLP in the 10% homogenate was high enough to produce some activation of the enzyme during preparative procedures. This was supported by the observation that the degree of saturation of the enzyme was significantly reduced when a 2% homogenate was prepared. When ATP was used to prevent binding of PLP in homogenates of freeze-blown brain, the degree of saturation of GAD fell further. Results suggested that the degree of saturation of GAD by its cofactor in vivo is at most 34%. Therefore, the enzyme in conventionally prepared homogenates is at least 2.4 fold more saturated than in vivo. The initially recorded high level of saturation (83%) can be attributed to both a postmortem in vivo increase and an in vitro activation occurring during preparative procedures. These studies suggest that factors affecting the binding of PLP to GAD may be important in regulating or influencing the rate of GABA synthesis.

1134 DIFFERENCES IN PRIMATE BRAIN REGIONS IN RELATIVE POTENCY FOR ANTAGONISM OF DOPAMINE-STIMULATED ADENYLATE CYCLASES BY NEUROLEPTIC DRUGS AND POSSIBLE IMPLICATIONS FOR LOCALIZATION OF ANTIPSYCHOTIC ACTIVITY. R.K. Mishra*, M. H. Makman*, H.S. Ahn*, B. Dvorkin*, S.G. Horowitz*, E. Keehn* and C. Demirjian*. (SPON: R. Katzman). Albert Einstein Col. Med., Bronx, N.Y. 10461.

Cebus and rhesus monkey frontal cortex (FC) contain adenylate cyclase (AC) stimulated by dopamine (DA) but in certain respects differing in responsiveness to DA agonists and to neuroleptic drug antagonists from the more "typical" DA-stimulated AC of caudate nucleus (CN) and also from that in anterior limbic cortex (ALC) (Ahn et al., Brain Res., 1976, in press). Additional evidence for heterogeneity of primate DA receptors has now been obtained by examination of the responsiveness to neuroleptic drugs of monkey ALC, FC and CN. Relative potencies for antagonism of DA-stimulated AC of homogenates were examined with fluphenazine as reference. Absolute sensitivity to both agonists and antagonists was greater in ALC than in FC. Relative potency of clozapine was somewhat greater in ALC than in CN and much greater in ALC than in FC. Pimozide was weaker in FC than ALC. It is concluded: (1) that there exist distinguishable types of DA-receptors associated with AC in the primate central nervous system; (2) that of the regions studied here and also in other studies of retina, the ALC contains DA-stimulated AC with sensitivity and relative responsiveness to neuroleptic drugs which best fits the antipsychotic activity of these drugs (although clozapine is more potent than predicted both in ALC and CN); and (3) that regional differences in relative potency of neuroleptic drugs for inhibition of DA-stimulated AC may explain at least to some extent, the reported partial dissociation of relative and antipsychotic potency from relative potency for production of certain side effects.

1135 EFFECTS OF STRESS DURING PREGNANCY ON CATECHOLAMINES IN DISCRETE BRAIN REGIONS. John A. Moyer*, Lorraine R. Herrenkohl and David M. Jacobowitz. Lab. Clin. Sci., NIMH, Bethesda, MD. 20014.

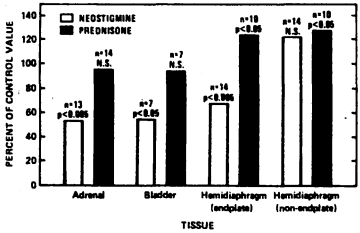
The effects of prepartal stress (PS) on catecholamine (CA) concentrations in twenty-three discrete brain areas associated with norepinephrine (NE) or dopamine (DA) containing pathways in the brain were examined by combining a microdissection technique for removal of individual brain regions with a sensitive radioenzymatic assay for NE and DA. Pregnant rats were exposed to stresses of heat, bright light, and restraint during the third trimester of pregnancy and compared to controls of stressed nonpregnant, unstressed pregnant, and unstressed nonpregnant females.

The most significant comparisons were found between the PS and unstressed nonpregnant states. Several regions innervated by the ventral ascending bundle showed the most consistent changes as a function of PS. PS reduced steady-state NE by about the same percentage in the N. preopticus medialis, the medial forebrain bundle, anterior hypothalamus, and the N. interstitialis stria terminalis. PS also selectively increased NE in the N. accumbens. Most of the structures innervated by the dorsal bundle showed no significant changes in steady-state CA as a result of PS. PS significantly decreased steady-state DA in the median eminence and increased steady-state DA in the N. amygdaloideus corticalis.

Locations of CA changes coincide in several brain areas with the localization of estrogen-concentrating neurons and with brain areas involved with gonadotrophin release and the neuroendocrine regulation of sexual behavior. This correlation suggests a common locus for sexual-hormonal and stress-induced activity.

1136 BINDING OF α-BUNGAROTOXIN IN DIAPHRAGM, ADRENAL, AND BLADDER FROM NEOSTIGMINE AND PREDNISONE TREATED RATS. Mark Noble*, William W. Hofmann*, and John Peacock. Dept/Neurol., Stanford Univ Sch Med, Stanford CA 94305.

Chronic treatment of rats with neostigmine causes decreased binding of ^{125}I-α-bungarotoxin to the endplate region of diaphragm muscle (1). We have studied toxin binding in neostigmine treated rats to determine 1) if a similar neostigmine effect occurs at sites other than skeletal neuromuscular junctions and 2) if prednisone (used in treatment of myasthenia gravis) will counter the neostigmine effect. Data summarized in the figure show a significant reduction in toxin binding per mg organ weight in adrenals and bladders as well as diaphragm endplate zones in neostigmine treated but not prednisone treated rats relative to control rats (same number for each experimental group). Neostigmine and prednisone treatments cause a significant reduction in animal weight and, in the case of neostigmine, diaphragm weight is significantly reduced; other organs show no weight loss. Combined neostigmine and prednisone treated rats have a more severe weight loss than for neostigmine alone. Addition of prednisone does not offset reduced toxin binding in neostigmine treated rats in any of the 3 organs examined. The marked reduction in toxin binding to adrenals and bladders but not diaphragm non-endplate region suggests a specific binding interaction. However, on the basis of competition experiments with cholinergic ligands, the toxin binding site is substantially different than for skeletal muscle. (Supported by NS12151 from the NINCDS, NIH; Calif. Chapter of the Myasthenia Gravis Foundation. (1) Chang, C.C.et al.(1973)J.Physiol.,230:613-618.



1137 MICROIONTOPHORETIC EVIDENCE FOR TWO RECEPTORS FOR GABA IN CEREBRAL CORTEX OF RAT. Franklin H. Norris* and Robert C.A. Frederickson. The Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IND. 46206.

α-δ-Diaminovaleric acid (ornithine, ORN) and δ-aminovaleric acid (AVA) have not been implicated as neurotransmitter candidates, but microiontophoretic AVA and ORN both have a weak depressant action on single neurons in brain (Curtis et al., 1960), possibly by acting on γ-aminobutyric acid (GABA) receptors. We have examined the effects of microiontophoretically applied GABA, AVA, L-ORN, D-ORN and bicuculline on single neurons of cerebral cortex in rat, and have obtained evidence for the existence of two GABA receptors, one of which responds also to ORN and AVA. The order of potency of these depressant amino acids in cerebral cortex was GABA> AVA>L-ORN>D-ORN. Analysis of the total data revealed a bimodal distribution for the potency of GABA on cortical neurons, suggesting interaction with two populations of receptors - one responding to very low doses of GABA (firing rate reduced 50% by decreasing the retaining current to - 5 nA) and one requiring higher doses of GABA (50% depression of firing with ejecting currents of 25-50 nA). The GABA data was unimodal in the presence of bicuculline which, however, was not a particularly potent antagonist of GABA in the cerebral cortex. Many GABA depressions were not antagonized at all, and many only partially, by the maximum currents of bicuculline that could be applied without directly exciting the cells. The depressant effects of AVA, L-ORN and D-ORN could be antagonized by bicuculline at least as readily as could those of GABA. The data for AVA, L-ORN and D-ORN were unimodal, which suggests that these amino acids act on only one of the two populations of GABA sensitive receptors. We do not know at this time whether the two receptor populations are both GABA receptors or whether one may be a receptor for another neurotransmitter (eg. glycine, alanine or ACh) for which GABA has some affinity.

1138 COMPARATIVE STUDIES OF GABA UPTAKE IN THE TADPOLE AND FROG CENTRAL NERVOUS SYSTEM. M. Pacheco, S. Glusman and B. Haber. Dept. of Physiology, Centro de Investigación del IPN, México, D.F. and The Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Tx.

In the tadpole, the tail is inervated by the terminal portion of the spinal cord; following metamorphosis, the tail is reabsorbed, and the majority of the neuronal elements involved in innervation of the tail degenerated. The resulting structure in the adult frog is the filum terminale (FT), which consists largely of glial elements, some ependymal cells and very few neurons. We have previously shown that choline and GABA uptake is significantly higher in the FT than in any other part of the frog CNS. In striking contrast to the frog FT, the GABA uptake is identical in all portions of the tadpole spinal cord during early development. Prior to metamorphosis, the terminal portion of the tadpole spinal cord, which is destined to become the FT in the frog, shows an increase in the uptake of GABA coincident with the loss of neuronal elements. DABA and β-alanine have been suggested to differentially inhibit GABA transport in neurons and glia; both compounds exert identical inhibitory effects in the FT and spinal cord. Studies of the tadpole spinal cord during various stages of development provides a unique model in which to study the effects of a changing neuronal population on some properties of normal glia.

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1139 A REVISED METHOD FOR STUDYING THE KINETIC COMPARTMENTATION OF DOPAMINE IN RAT STRIATUM. Charles Paden* (SPON: Seth K. Sharpless). Dept. Psych., Univ. Colo., Boulder, CO 80309.

Javoy and Glowinski (J. Neurochem. 18: 1305, 1971) observed two phases of dopamine (DA) decline from the rat striatum following the i.p. injection of 200 mg/kg α-methyl-p-tyrosine methyl ester (αMpT). They proposed that the initial decline represented the "functional" compartment of newly synthesized DA while the later decline corresponded to the "main storage" compartment. Doteuchi, Wang and Costa (Mol. Pharmacol. 10: 225, 1974) have argued for a single compartment of striatal DA based on the rate of conversion of ^{3}H-tyrosine to ^{3}H-DA in the absence of synthesis inhibition.

Experiments using a revised isotopic method have been undertaken in an attempt to obtain further evidence regarding the compartmentation of striatal DA. ^{3}H-Tyrosine was injected i.v. into male rats followed 10 minutes later by an i.p. injection of αMpT (400 mg/kg). Animals were then sacrificed at 5 minute intervals. This dosage of αMpT resulted in greater than 90% inhibition of DA synthesis within 5 minutes. Since the DA specific activity in an open compartment approaches the mean specific activity of the precursor in an asymptotic manner ($1-e^{-kt}$), it is possible to predict that following exposure to a 10 minute pulse of ^{3}H-tyrosine the "functional" and "main storage" DA compartments as described by Javoy and Glowinski should achieve 54% and 6% of the mean precursor specific activity, respectively, resulting in 77% of the ^{3}H-DA versus only 23% of the total DA being located in the "functional" compartment. Thus the majority of ^{3}H-DA should disappear from the striatum with a half-life characteristic of the "functional" pool following blockade of synthesis with αMpT. Preliminary results indicate that while total striatal DA appears to decline biphasically as previously reported, the newly synthesized ^{3}H-DA does not appear to decline in a manner consistent with the above prediction.

1140 EFFECTS OF MORPHINE ON THE FROG SPINAL CORD. Ante L. Padjen. Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6

It is well known that morphine causes excitation and convulsions in certain animals. In frogs these effects are especially prominent occuring before any antinociceptive action (cf. Nistri et al., Brain Res.80:199, 1974). This phenomenon was analyzed on isolated superfused frog spinal cord. Synaptic and amino acid responses were recorded from spinal roots using sucrose gap technique. Morphine sulfate (MS) in conc. above 10^{-4}M selectively abolished dorsal root potential (DRP) evoked by ventral root stimulation and prolonged DRP and ventral root potential obtained by dorsal root stimulation. Responses to amino acids (recorded in the presence of 20 mM Mg^{++}) were also differentially affected by the same conc. of MS. Depolarizations of primary afferents (PA) and hyperpolarizations of motoneurons (MN) caused by β-alanine and taurine were markedly attenuated. MN hyperpolarization by glycine was abolished and often reversed to a depolarization. Responses to GABA and glutamate on PA and MN as well as depolarizing response of PA to glycine were unaffected. Similar actions were produced by levorphan, codeine, ethorphine and thebaine, as well as by naloxone and naltrexone which did not antagonize MS effects. At conc. up to 10^{-3}M none of the drugs directly affected membrane potential of MN or PA. These results suggest that the opiate induced convulsions in frog spinal cord are due to a strychnine-like antagonism of amino acid receptors (cf. Barker et al., J. Physiol., 245; 537, 1975) and unrelated to a stereospecific opiate receptor.

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1141 UPTAKE AND METABOLISM OF D AND L ISOMERS OF 5-HYDROXYTRYPTOPHAN IN RAT BRAIN. P.E. Penn* and W.J. McBride. The Institute of Psychiatric Research, Indiana University Sch. of Med., Indianapolis, IN. 46202.

When studying the effects of 5-hydroxytryptophan (5-HTP) as a precursor or label in the serotonergic system, the D,L form is frequently exogenously administered. The present study was designed to investigate the relative contribution of the D and L isomers to the uptake and metabolism of 5-HTP. Injections of D or L 5-HTP (25 mg/kg) were given intraperitoneally to male rats which were then killed at 15, 30, 45, and 60 minutes after injection. Endogenous levels of 5-HTP, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were measured in the telencephalon and in the brain stem (including diencephalon). In addition, the levels of norepinephrine (NE) and tyrosine (tyr) were measured in the telencephalon. Compared to vehicle controls, L-5-HTP significantly increased the levels in the telencephalon of 5-HTP, 5-HT and 5-HIAA at all time units. At 15 minutes this change was 2760% for 5-HTP, 157% for 5-HT and 788% for 5-HIAA. Similar changes were found in the brain stem. In contrast D-5-HTP increased the levels in the telencephalon of 5-HTP (497%) and 5-HIAA (166%) at 15 minutes only and no increase was obtained for 5-HT at any time. Consequently at 15 minutes the contribution of the D isomer compared to the L isomer to the uptake of 5-HTP is 18% and to the metabolism to 5-HIAA is 21%. No marked change was seen in the levels of NE or Tyr after the injection of either isomer. The data indicated that the L isomer of 5-HTP was more readily taken up and metabolized in the telencephalon than was the D isomer. (Supported in part by NIMH Grants MH-03225-17 and MH-05986-20).

1142 RELEASE OF PHYSIOLOGICALLY LABELLED AMINO ACIDS FROM GUINEA PIG CEREBRAL CORTEX SLICES. S. J. Potashner* (Spon: A.-T. Tan). Dept. Research in Anaesthesia, McGill University, Montreal, Quebec, Canada.

Evoked release of putative amino acid transmitters has been widely studied using exogenous labelled amino acids. The latter, after transport into nervous tissues, are not always distributed as are their endogenous counterparts. To approach the physiological more closely, the evoked release of endogenous putative amino acid transmitters, labelled from [U-^{14}C]D-glucose, was studied using an *in vitro* superfusion technique. Adequate labelling of endogenous putative amino acid transmitters could be achieved using carrier-free [U-^{14}C]D-glucose. Spontaneous efflux of endogenous ^{14}C-aspartate, ^{14}C-glutamate, and ^{14}C-γ-aminobutyrate (GABA) was 0.1-0.2% (of the tissue amino acid) per minute, while that of ^{14}C-alanine and ^{14}C-threonine-serine-glutamine (unseparated) was 20-30%/min. Electrical field stimulation (rectangular biphasic pulses, 20 mA, 5 ms, 100/s) transiently increased the release of ^{14}C-aspartate, -glutamate and -GABA to 7-11X the spontaneous level. Release of ^{14}C-alanine and -threonine-serine-glutamine was increased to only 1.5-2.5X. The release of ^{14}C-alanine and -threonine-serine-glutamine was insensitive to Ca^{++}-removal, but 70-90% of ^{14}C-aspartate, -glutamate and -GABA release was Ca^{++}-dependent. Spontaneous release of exogenous ^{14}C-aspartate, ^{3}H-GABA, ^{14}C-αAIB and ^{14}C-urea was 0.7, 0.2, 2.5 and 14.4%/min, and stimulation increased release to 2X (at peak), 4X, 1.7X and 1X respectively. 40-50% of the release of exogenous ^{14}C-aspartate and ^{3}H-GABA was Ca^{++}-dependent. ^{14}C-αAIB release was Ca^{++}-independent. Evoked release of endogenous putative transmitters - aspartate, GABA, and probably glutamate - differs from that of their exogenous counterparts , as well as endogenous alanine and threonine-serine-glutamine.

(Supported by the Medical Research Council of Canada).

1143 EFFECT OF MORPHINE IN VIVO ON RELEASE OF [^{3}H]-ACETYLCHOLINE FROM RAT STRIATAL SYNAPTOSOMES IN VITRO. D.A. Redburn, T. Rujirekagulwat*, T. Cope*, and G.C. Rosenfeld*. The University of Texas Medical School at Houston, Houston, Texas 77025.

Morphine sulfate (M) (50mg/kg) or saline (S) was administered (s.c.) to male Sprague-Dawley rats (TIMCO, Houston). One hour postinjection, striatal synaptosomes were isolated and incubated with [^{3}H]-choline (0.25μM) for 10 minutes at 37°C. The synaptosomes were collected on glass fiber filters (WHATMAN, GF/A) and superfused with a modified Ringer's solution containing depolarizing levels of K^{+}(56mM) either in the presence or absence of 3mM Ca^{++}. The amount of [^{3}H]-acetylcholine (ACh) released from K^{+} depolarized synaptosomes, both in the presence and absence of Ca^{++}, was increased 76% by M pretreatment. However, the in vitro uptake rate and total accumulation of [^{3}H]-choline in striatal synaptosomes were also increased by M pretreatment. Thus, when Ca^{++}-dependent release of [^{3}H]-ACh is expressed as a percent of total [^{3}H]-choline accumulation, there is no significant difference between M and S treated animals. All of the effects of M were completely blocked by simultaneous administration of naloxone (1mg/kg). Direct addition of M (10^{-7}-10^{-4}M) to synaptosomes from S treated animals was without effect on either release or uptake. Drug-induced changes of cholinergic activity have recently been shown to be correlated with changes in the in vitro rate of choline uptake (Simon, J.R. and Kuhar, M.S.; Nature 255:163, 1975). Our results indicate, therefore, that although M has no long-term effect on cholinergic release mechanisms as measured in vitro, it may indirectly stimulate cholinergic neurons within the striatum. (Supported in part by USPHS Grant DA-00926.)

1144 IMMUNOCYTOCHEMICAL LOCALIZATION OF GAD IN SOMATA AND DENDRITES OF GABAERGIC NEURONS FOLLOWING COLCHICINE TREATMENT. Charles E. Ribak* and James E. Vaughn. City of Hope Nat. Med. Ctr., Duarte, CA. 91010.

Previous immunocytochemical studies have localized glutamate decarboxylase (GAD) in axon terminals, but the somata and dendrites of neurons giving rise to these GAD-positive axon terminals lacked reaction product (McLaughlin, et al., Br. Res. 76:377, 1974). However, in a recent study of the olfactory bulb, GAD has been detected in the somata and dendrites of granule and periglomerular cells (Ribak, et al., Anat. Rec. 184:512,1976). Therefore, GAD in the somata and dendrites of these GABAergic neurons can be visualized with the same anti-GAD sera that was used to identify GAD in axon terminals. The neurons showing somal staining in the olfactory bulb have numerous presynaptic dendrites and thus differ from those neurons which did not show somal staining in previous studies. An inference from these observations is that neurons which lack presynaptic dendrites may have GAD in their somata but in a low, undetectable concentration relative to that in their terminals because newly synthesized GAD may be rapidly transported to axon terminals. This inference was tested by using colchicine to block axonal transport thereby allowing for an accumulation of GAD in somata and dendrites. Injections of 50-100 µg of colchicine (10 µg/µl saline) were made into the cerebellum and hippocampus of rats. Following a 24 hr. survival time, the injected brains were processed for GAD immunocytochemistry. Marked accumulations of GAD-positive reaction product were observed in the somata and dendrites of Purkinje, Golgi, basket and stellate cells in the cerebellum and in the basket and other short axon cells of the hippocampus. None of these somata exhibited reaction product in preparations that were not pretreated with colchicine. Thus, the use of colchicine in combination with GAD immunocytochemistry allows for a direct identification of neuronal types which probably use GABA as their neurotransmitter. (Supported by USPHS grants #NS12116 and #NS1615).

1145 INFLUENCE OF LHA CHOLINERGIC STIMULATION ON THE PLASMIC RENAL FLOW AND THE RATE OF GLOMERULAR FILTRATION. Wilson A. Saad, Cincinato R. Silva Neto*, Luiz A. de A. Camargo*, José A. Rodrigues* and Miguel R. Covian*. Dept. of Physiol., Sch. of Dent. and Pharm., 14.800 Araraquara, SP, Brasil.

Cholinergic stimulation of the lateral hypothalamic area (LHA), especially at the level of the anterior hypothalamus, induces natriuresis and kaliuresis with a decrease in urinary volume reaching its lowest level between 40 and 60 min. Arterial pressure, plasmic renal flow (PRF) and rate of glomerular filtration (RGF) were determined at the 40 min time mark in order to study possible alterations in renal haemodynamics caused by cholinergic stimulation. Holtzman rats anesthetized with nembutal were submitted to a continuous intravenous jugular infusion of a 3% glucose and 0.25% sucrose solution (250 m O sm/l) at the rate of 0.51 ml/min. Urine samples were collected through a bladder tube and blood samples through a tube in the femoral artery. The plasmic renal flow and the rate of glomerular filtration dropped slightly 40 min after cholinergic stimulation. The level of plasmic sodium obtained during the experimental period was lower than the level in the control period; the quantity of filtered sodium was lower during the experimental period. An injection of carbachol in the LHA induced an increase in the excretion of the Na^+ electrolyte; however, the percentage of tubular reabsorption did not show any significant difference. The increase in sodium excretion was accompanied by a decrease in urinary volume. The arterial pressure values did not change significantly between the two periods. It was concluded that cholinergic stimulation of the LHA caused a significant reduction in RGF and PRF, and that, in spite of the lower amount of filtered sodium, there was an increase in natriuresis, which, according to the results, seems to be independent of renal haemodynamic alterations. (Supported by Grant Biol. 75/740 FAPESP).

1146 IN VIVO DEMONSTRATION OF SODIUM-DEPENDENT HIGH-AFFINITY HIPPOCAMPAL CHOLINE UPTAKE. G. M. Samaras* and J. F. Contrera. Univ. of Maryland, College Park, MD. 20742.

A general methodology for measuring neurotransmitter uptake in the living mammalian brain has been developed. The method employs in vivo iontophoretic administration of a neurotransmitter or precursor followed by subcellular fractionation and biochemical analysis of metabolites. We report the use of this method to study choline uptake in vivo in the dorsal hippocampus. In vitro methods indicate that pentobarbital, oxotremorine, scopolamine and potassium depolarization affect only the sodium-dependent high-affinity choline uptake system in the hippocampus. We have found that pentobarbital (65 mg/kg), oxotremorine (0.75 mg/kg) and medial septal area lesions (3 day) yield large decreases in choline uptake in vivo (47%, 28%, and 58% respectively) relative to saline controls. Conversely, scopolamine (5 mg/kg) and iontophoretically applied potassium yield large increases in choline uptake in vivo (36% and 39% respectively) relative to saline control. We feel these data indicate that the sodium-dependent high-affinity hippocampal choline uptake system is functional and not saturated in vivo.

1147 MICROIONTOPHORETIC EVALUATION OF HISTAMINE AND HISTAMINE RECEPTOR ANTAGONISTS ON FELINE VESTIBULAR NEURONS. J. Satayavivad* and E. B. Kirsten, Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.

Iontophoresis of histamine (25-100 nA) was evaluated on spontaneously firing neurons in decerebrate, cerebellectomized cats. Histamine produced a marked inhibition of firing rate in the majority of cells (64%; n=109 cells). In contrast, an excitatory response was observed in 24% (n=42) of the cells tested. Inhibitory responses to histamine were characterized by a very rapid decrease and recovery in spike discharge. Histamine excitation had a delayed onset with the peak excitatory response occurring after cessation of histamine ejection current and with a prolonged afterdischarge. The effectiveness of H_1- and H_2- antagonists in blocking histamine-induced iontophoretic responses was evaluated by using the H_1-antagonist, diphenhydramine (25-100 nA) and the H_2-antagonists, metiamide (25-50nA) and cimetidine (25-50 nA).

Iontophoresis of diphenhydramine had a direct depressant action on the vestibular neuronal membrane and was ineffective in blocking histamine-induced inhibitory responses. Cimetidine often produced a depression of spontaneous discharge without antagonizing either the excitatory (n=10) or the inhibitory (n=9) responses to histamine. Metiamide was effective in blocking the inhibitory response to histamine (n=22) but not the excitatory response (n=5). These results suggest that the inhibitory and excitatory responses to histamine are mediated by different receptors. The histamine inhibitory response appears to be mediated by an H_2-receptor while the excitatory response remains undefined. (Supported by NINDS Grant NS-11858 and The Rockefeller Foundation).

1148 EFFECTS OF ADENINE NUCLEOTIDES AND GLUTAMATE ON THE ACTIVITY OF RAT BRAIN GLUTAMATE DECARBOXYLASE. B. Seligmann*, D.E. Brockman*, L.P. Miller*,and D.L. Martin* (SPON: A.T. Campagnoni). Dept. Chemistry, Univ. Maryland, College Park, 20742 and Lab. Neuropharmacol, NINCDS, Bethesda, MD 20014.

Glutamate decarboxylase (GAD,EC 4.1.1.15) in a high-speed supernatant of rat-brain homogenate retains a high proportion of its activity following exhaustive dialysis indicating that the cofactor, pyridoxal-5'-phosphate, (PLP) is tightly bound to the enzyme in the absence of substrate. When the dialyzed enzyme was assayed in the absence of added PLP, the reaction rate decreased rapidly with time. This effect was strongly dependent on the glutamate concentration suggesting that glutamate promotes the dissociation of PLP from the enzyme. This conclusion is supported by the observation that enzymatic activity can be restored by the addition of PLP. In the presence of 100 μM PLP, the reaction rate was constant for over 60 min. When assayed in the absence of PLP, the dialyzed enzyme was not significantly inhibited by adenine nucleotides indicating that adenine nucleotides do not promote the dissociation of PLP from the enzyme. The addition of ATP (0.25-8 mM) to a reaction mixture containing 10 μM PLP and sufficient glutamate to promote the dissociation of PLP from the enzyme resulted in progressive inactivation of the enzyme. This effect was also observed with ADP and the Mg^{2+} complexes of ATP and ADP but did not occur with AMP. The effects of adenine nucleotides were reduced by the addition of 5-10 mM P_i and could be completely overcome by 500 μM PLP. Our interpretation of these results is that adenine nucleotides inhibit GAD by blocking association of PLP with the enzyme. These findings suggest that PLP and the adenine nucleotides may be involved in the regulation of GABA synthesis. Supported in part by Grant No. RO3-MH-26491-01 from USPHS.

1149 (^{3}H) DOPAMINE UPTAKE INTO SYNAPTOSOMES FROM RAT BRAIN: EFFECTS OF TRYPSIN. Virendra K. Sharma,* Lily S. Kung* and Shailesh P. Banerjee. Dept. of Pharmacology & Toxicology, Univ. of Rochester, Rochester, New York 14642.

Exposure of synaptosomes derived from rat brain cortex to 10 μg/ml of trypsin for 15 min at 37°C, decreased the initial rate of Na^+-dependent (^{3}H) dopamine (DA) uptake to about 50% of the control. Again a concentration of 50 μg/ml of trypsin abolished 60% of Na^+-dependent (^{3}H) DA uptake in 5 min. The Na^+-dependent (^{3}H) DA uptake in synaptosomes derived from cortex as well as from corpus striatum exhibited similar sensitivity towards trypsin. On the other hand, there was little or no alteration in Na^+-independent accumulation of (^{3}H) DA due to the prior exposure of synaptosomes to trypsin. The kinetic analysis indicated that the inhibition of Na^+-dependent (^{3}H) DA uptake in cortical as well as in striatal synaptosomes by trypsin was non-competitive, which decreased the maximal velocities with no alterations in apparent affinities of these two populations of synaptosomes for (^{3}H) DA uptake. Although (^{3}H) DA may be accumulated in non-dopaminergic neurons by Na^+-dependent, high affinity uptake system, the dopamine-containing nerve endings are predominantly concentrated in the straitum. Since the Na^+-dependent (^{3}H) DA uptake into cortical and straital synaptosomes exhibited similar sensitivity to inhibition by trypsin, this proteolytic enzyme appears to decrease (^{3}H) DA uptake in dopaminergic neurons besides affecting other monoaminergic nerve endings. (Supported by a grant HL 18185 from the N.I.H. and a grant-in-aid from the American Heart Association.)

1150 THE EFFECTS OF INJECTIONS OF CHOLINE, DOPA, 5-HYDROXYTRYPTOPHAN, TRYPTOPHAN, AND TYROSINE ON THE LEVELS OF EIGHT PUTATIVE NEUROTRANSMITTERS IN RAT WHOLE BRAIN. P.A. Shea*, J.E. Smith, J.D. Lane*, and W.J. McBride (SPON: F.D. Walker). The Institute of Psychiatric Research and Depts. of Biochemistry and Psychiatry, Indiana University Medical School, Indianapolis, Indiana 46202.

Male Wistar rats (200-300g) were injected i.p. with either 300mg/kg of L-tryptophan (Try), 50mg/kg of D, L-5-hydroxytryptophan (5-HTP), 300 mg/kg of L-tyrosine (Tyr), 100 mg/kg of L-DOPA or 100 mg/kg of choline and killed by the near-freezing method at 15, 30 or 45 minutes after injection. The whole brains were removed in a refrigerated cold box (-10°C), pulverized under liquid nitrogen and acetylcholine (ACh), norepinephrine (NE), serotonin (5-HT), dopamine (DA), asparate (Asp), glycine (Gly), glutamate (Glu) and GABA determined by the method of Smith et al. (Anal. Biochem. 64, 149, 1974). The content of ACh was not affected by any of the injected compounds. Compared to vehicle controls, choline and L-DOPA caused a decrease in the levels of all the putative amino acid neurotransmitters at various time intervals. No effect on the levels of these compounds was observed after injections of 5-HTP. Injections of Try caused a decrease in the levels of Asp, Gly, and Glu while injections of Tyr caused a decrease in the levels of Asp and GABA. The level of NE was decreased 30 min after injections of Try and 5-HTP and 15 min after injections of Tyr and L-DOPA, whereas the content of NE increased at 45 min after injections of 5-HTP, L-DOPA and Tyr. The content of DA increased at all three time points after injections of L-DOPA but not after Tyr. Levels of 5-HT were increased after injections of Try and 5-HTP. These data indicate that precursors to specific neurotransmitters can affect the levels of other putative neurotransmitters in rat whole brain. (Supported in part by PHS Grants MH-03225-17 and MH-10695)

1151 THREE-DIMENSIONAL FLUOROMETRIC DETERMINATION OF LSD-BINDING. Jean C. Shih and Joon Rho*. School of Pharmacy, USC, Los Angeles, California 90033.

Serotonin-binding protein (SBP) from hypothalamus synaptosomal membrane fragments was isolated by affinity column chromatography, in which serotonin was attached to Sepharose via a diazonium derivative and protein was eluted by D-Lysergic acid diethylamide (D-LSD) or Chlorimipramine (CIP) (J.C. Shih, et. al., Adv. Biochem. Psychopharm., Vol. 11, 101-104, 1974). The binding property of SBP toward D-LSD or CIP has been further investigated by a highly sensitive "three-dimensional" fluorescence spectroscopy. This instrument records simultaneously the activation and fluorescence spectra, and plots fluorescence intensity level by a series of isointensity contours. The optical system has been designed for use with a highly sensitive trialkalic photocathode tube cooled to -20°C to reduce its thermal noise. With this instrument, we are able to study the specific interaction of D-LSD (or CIP) with SBP. D-LSD exhibits maximum fluorescence at 450nm with excitation maximum at 325nm in neutral pH, while SBP-bound D-LSD shifted its excitation and fluorescence peaks to 400 and 500nm respectively. Chlorimipramine (CIP) also shifted its excitation and fluorescence peaks substantially when it was bound to SBP. The fluorescence spectral shift of bound-D-LSD (or CIP) indicated the drug-protein binding much more specifically than conventional binding studies using radioactive drug. Furthermore, with three-dimensional fluorescence techniques we are able to determine the extent of bound-LSD (or bound-CIP), as well as its free form in the same sample. (Supported by grants from Eli Lilly Company, Abbott Laboratory and USPHS, NCI, Grant CA 14089 to LAC/USC Comprehensive Cancer Center).

1152 BIOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES OF CYCLIC NUCLEOTIDES AND THEIR EFFECTS IN THE RAT SUPERIOR CERVICAL GANGLION. P. Shinnick-Gallagher*, B .J. Williams*, and J. P. Gallagher. Dept. of Pharmacology and Toxicology, Univ. Texas Medical Br., Galveston, Texas 77550.

An analysis of biochemical and electrophysiological events occurring during and as a result of ganglionic transmission from individual rat superior cervical ganglia has been carried out. Ganglia were maintained in vitro by perfusion with Krebs-Ringer solution and assayed for cyclic adenosine monophosphate (cAMP) using the Gilman protein method. Comparisons were made between the resting levels of the nucleotides and levels of the nucleotides after stimulation at 10 Hz for 2 min. Tissue c-AMP levels were measured after incubation with dopamine, with acetylcholine (ACh), with the phosphodiesterase inhibitor, methyl isobutyl xanthine (MIX) and various combinations. Orthodromic stimulation increased ganglionic cAMP, but incubation with ACh did not. cAMP accumulation after dopamine was enhanced by MIX. Intracellular injection of cAMP into individual ganglion cells using double-barrelled microelectrodes produced a concentration dependent, reversible increase in conductance. On the other hand, extracellular perfusion with dibutyryl-cAMP (2.5 mM) produced no changes of the resting membrane potential, passive membrane properties or action potentials evoked by orthodromic or direct stimulation. Similar results were obtained following cyclic guanosine monophosphate (cGMP) injection, i.e. a concentration dependent, reversible increase in conductance. Although cyclic nucleotide levels may be altered as a result of electrical or drug stimulation, the present results suggest that these changes cannot be clearly related to current theories for the role of cyclic nucleotides in ganglionic transmission.

1153 HUNTINGTON'S DISEASE (HD): DETERMINATION OF PLASMA NOREPINEPHRINE (NE) AND DOPAMINE-β-HYDROXYLASE (DBH). Ira Shoulson, Michael Ziegler, and Raymond Lake. NINCDS & NIMH, NIH, Bethesda, MD. 20014.

Abnormalities of vasoregulatory activity and plasma DBH, the immediate synthetic enzyme for NE, have been reported in patients with HD.

Plasma concentrations of NE and DBH were measured by a sensitive radioenzymatic technique in 9 untreated HD patients and 9 age and sex matched control subjects during supine rest, after standing and following a standard isometric hand grip. Circulating NE and DBH under resting conditions were significantly lower in the HD patients; but control and HD subjects demonstrated substantial and similar increases in plasma NE and DBH following standing and isometric stimulation. Differences in pulse rate or blood pressure between the two groups were not observed at rest or following the vasomotor manipulations. In general, plasma NE reflected vasoregulatory changes more accurately than DBH in both groups.

In summary, resting plasma NE and DBH concentrations are significantly lower in HD patients than controls despite similar baseline pulse rate and blood pressure profiles. The responses to standing and isometrics, both neurochemical and vasomotor, are virtually identical in both groups. The foregoing observations suggest that the CNS pathology in HD may contribute to resting neurochemical changes in peripheral NE without significantly altering sympathetic vasoregulatory responsiveness.

1154 MORPHINE-LIKE PEPTIDE, ENKEPHALIN: RADIOIMMUNOASSAY AND DISPOSITION IN NORMAL AND DRUG ALTERED MAMMALIAN BRAIN. Rabi Simantov* and Solomon H. Snyder (Spon. J. Brady) Depts. Pharmacol. and Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD. 21205

A morphine-like factor has been identified as a mixture of two peptides methionine-enkephalin (tyr-gly-gly-phe-met, m-enk) and leucine enkephalin (tyr-gly-gly-phe-leu, l-enk) using bioassay in pig brain (Hughes et al. Nature 258:577, 1975) and by opiate receptor competition in bovine brain (Simantov and Snyder, Life Science 18:781, 1976). Pig brain contains four times more m-enk than l-enk while the ratio is reversed in calf brain. Because washed brain membranes degrade enkephalins under conventional opiate receptor binding conditions, techniques were developed which stabilize enkephalins. Immunizing guinea pigs with synthetic m-enk and l-enk coupled to protein elicited specific antibody production, permitting development of a sensitive and selective radioimmunoassay. Subcellular, regional and phylogenetic distributions of m-enk and l-enk were evaluated by radioimmunoassay and/or competition for opiate receptor binding. Enkephalin levels parallel the distribution of the opiate receptor with localization intracellularly in synaptosomes, regionally in limbic areas, and phylogenetically in all vertebrates but no invertebrates. Enkephalin levels double in brains of rats rendered physically dependent on morphine and return to normal as abstinence symptoms subside.

1155 INTERACTION OF LIMBIC STRUCTURES IN THE CONTROL OF CHOLINERGIC RAGE. Cornelis L. J. Stokman and Murray Glusman. Dept. of Behavioral Physiology, N.Y.S. Psychiatric Institute, New York City, N.Y. 10032

Aggressive reactions in cats can be induced by local micro-injections of acetylcholine or carbachol into the hypothalamus or mesencephalic central gray. These dose-dependent responses can be blocked by prior injection of atropine in the same site. Cholinergic rage is also sensitive to pharmacologic manipulation of other limbic sites. Atropine injected into the midbrain central gray blocked carbachol-induced aggression in the hypothalamus. However, atropine injected into the hypothalamus did not prevent carbachol-induced aggression in the central gray, suggesting that the functional integrity of the midbrain cholinergic neurons is necessary for aggression induced by cholinergic stimulation of the diencephalon, but not *vice versa*. The pharmacologic findings closely parallel data obtained from the same structures using electrical stimulation and electrolytic lesions to elicit and block aggressive responses.

1156 CORRELATED EFFECTS OF ACETYLCHOLINE (ACh) AND CYCLIC-GMP (cGMP) ON INPUT RESISTANCE (Rm) OF NEOCORTICAL NEURONS IN AWAKE CATS. B. Swartz* and C.D. Woody. UCLA Medical Center, Los Angeles, California 90024.

Rm increases transiently in neurons of sensorimotor cortex after extracellular iontophoresis of ACh (Krnjevic et.al. J. Physiol. 1971). Woody et.al. (Fed. Proc. 1974) confirmed these results and noted that when ACh application was paired with current injection sufficient to fire penetrated neurons, persistent rather than transient changes in Rm occurred. Analogous changes in Rm were seen in response to intracellular iontophoresis of cGMP (Woody et.al. Fed. Proc. 1976). Rm did not increase after "control" iontophoresis of saline for ACh or 5'GMP for cGMP. In the present study the intracellular recording barrel contained 5 μM cGMP in 1.4 M K^+citrate. The two adjacent extracellular barrels contained 2M ACh and 1M atropine respectively. ACh was applied initially (+400 nA, 30 sec). Three or more minutes later, atropine was applied (+400 nA, 30 sec), and 30 sec later the ACh trial was repeated to determine whether observed responses could be blocked by atropine. Finally cGMP was intracellularly iontophoresed (-2nA, hyperpolarizing, 30 sec). Rm was measured during iontophoresis of all agents and for at least 3 min. thereafter by both differential spike height and bridge imbalance techniques. 18 units with average spike amplitude of 44 mV and membrane potential of 47 mV were studied in this way. Of these, 8 units (44%) responded to ACh with an increase in Rm. In all of these units no increase in Rm occurred after atropine during the second trial of ACh, whereas all subsequently showed an increase in Rm when cGMP was given. No units responded to ACh without showing a response to cGMP. These results support the hypothesis that cGMP could be a second messenger for muscarinic, ACh-induced changes in membrane conductance in mammalian neocortex. Transience or persistence of these changes in Rm depended on current-induced discharge of the penetrated neuron as per Woody et.al. (op.cit.). (Supp. by USPHS HD-05958, HD-04612)

1157 PRESYNAPTIC EFFECTS ON [^{3}H]ACETYLCHOLINE RELEASE FROM HIPPOCAMPAL SLICES. J.C. Szerb, P. Hadházy*, and J.D. Dudar*, Dept. Physiology and Biophys., Dalhousie U., Halifax, N.S.

In order to study factors that affect the in vitro release of [^{3}H]ACh from the septo-hippocampal cholinergic pathway, slices of rat hippocampus were incubated with 1 μM [^{3}H]choline, then superfused with 10 μM hemicholinium-3. The spontaneous and evoked (1 Hz pulses) release of radioactivity was measured. The spontaneous efflux was halved and the evoked release completely eliminated one week after lesioning the medial septum, indicating that cholinergic fibers are the source of the evoked release of the label. Muscarinic agonists in the presence of 2 nM atropine reduced, while antagonists in the presence of 0.8 μM oxotremorine increased the rate constant of the evoked release in a dose-dependent manner. The 50% effective concentration (in μM/l) were: oxotremorine 0.3; carbamylcholine 2; atropine 0.02; quinuclidinyl benzilate 0.01; scopolamine 0.01. Results suggest that muscarinic agonists are more effective while antagonists are about 10 times less effective in producing a presynaptic effect on ACh release, as compared to their affinity to muscarinic receptors in brain homogenates which according to Yamamura and Snyder (Brain Res., 1974, 78:320) are localized postsynaptically. (Supported by MRC of Canada.)

1158 GABA-STIMULATED FLUX OF RADIOLABELED CHLORIDE IONS IN CRAYFISH MUSCLE. M.K. Ticku* and Richard W. Olsen. Department of Biochemistry, University of California, Riverside, CA 92502, USA.

Gamma-aminobutyric acid(GABA)selectively increased chloride permeability in isolated strips of crayfish abdominal muscle. Uptake of $^{36}Cl^-$ by muscle was followed at room temperature in Van Harreveld's solution. The equilibration showed a $t_{\frac{1}{2}}$ of 2-3 min and a total Cl^- space of 700 ml/kg wet wt. tissue. Uptake for exactly 15 sec gave a Cl^- space of 131±4 ml/kg (n=60)which was significantly increased($p<0.05$)by GABA($\geq$200uM)to 173±5 ml/kg(n=30). Stimulation of Cl^- uptake by GABA was dose-dependent with 50% of maximal response at 40uM. Muscimol, a GABA agonist,gave the same maximal response as GABA but at lower doses($\geq$50uM); γ-amino,β-hydroxy GABA(GABOB),$\geq$1mM, a partial agonist, increased $^{36}Cl^-$ uptake at 15 sec only slightly. Stimulation by GABA was specific for Cl^-: uptake of radiolabeled sucrose, inositol, or propionate were not affected. GABA-stimulated chloride uptake was inhibited by the antagonists picrotoxinin, bicuculline, β-guanidinopropionate, and γ-guanidinobutyrate(2mM).Picrotoxinin which did not affect control Cl^- uptake, inhibited GABA-stimulated Cl^- uptake in a dose-dependent fashion with 50% inhibition at 4-5uM. These results confirm electrophysiological observations of the increased permeability of the postsynaptic membrane to chloride ions in response to the inhibitory neurotransmitter GABA, and provide a test-tube assay for mechanism and structure-function studies of GABA receptor-ionophores.

Supported by NSF GB42032 and NIH NS12422.

1159 ^{125}I-NEUROTENSIN BINDING TO BRAIN MEMBRANES. George R. Uhl*, James P. Bennett, Jr., and Solomon H. Snyder (SPON: Malcolm S. Preston). Dept. Pharmacology and Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD. 21205

Neurotensin (NT) is a tridecapeptide (< Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH) isolated from bovine hypothalamic extracts and synthesized by Carraway and Leeman (JBC 248:6854-6861; JBC 250:1907-1918). Peripherally administered NT produces hypotension, increased vascular permeability, pain sensation, increased hematocrit, cyanosis, morphine-inhibitable stimulation of ACTH secretion, increased LH secretion, increased FSH secretion, and hyperglycemia. In vitro smooth muscle effects of NT include contraction of the estrous rat uterus, contraction of the guinea pig ileum, and relaxation of the rat duodenum.

^{125}I-NT binds saturably, reversibly, with high affinity (K_D approx. 3 nM), and with marked regional variation to membranes prepared from rat, calf, and human brains. Sequence fragments of NT displace ^{125}I-NT from brain and uterine membranes with differential potencies that parallel their relative potencies in peripheral systems. Other unrelated peptides and neurotransmitter candidates tested show less than 50% displacement of ^{125}I-NT binding at 1μM. Our evidence suggests that ^{125}I-NT binding may represent interaction with a physiologically-relevant receptor; implications of this finding in support of a possible synaptic role for NT in mammalian brain will be discussed.

1160 EFFECT OF CHOLINE ON TYROSINE HYDROXYLASE (TOH) ACTIVITY IN THE RAT CAUDATE NUCLEUS. Ismail H. Ulus,* Michael C. Scally* and Richard J. Wurtman. MIT, Cambridge, MA 02139.

We have previously shown that choline administration accelerates brain acetylcholine (ACh) synthesis and raises ACh levels (Cohen and Wurtman, *Life Sci. 16*: 1095, 1975; Cohen and Wurtman, *Science 191*: 561, 1976). To determine whether this precursor-induced increase is associated with increased release of the transmitter, we examined the effect of choline on TOH activity in the rat caudate nucleus, a brain region known to have interactions between cholinergic and dopaminergic neurons. Two hours after choline administration (60 mg/kg choline chloride) caudate TOH activity increased from 1.91 $\pm$ 0.11 to 2.28 $\pm$ 0.09 nmol CO_2/hour/mg protein; two hours after a larger dose (120 mg/kg), caudate TOH activity was 2.60 $\pm$ 0.20. Atropine sulfate (40 mg/kg) administration had no effect on TOH activity but completely blocked the choline-induced rise. This observation indicates that choline administration increases the flow of information across central cholinergic neurons.

1161 SYNAPTIC CONNECTIONS MEDIATED BY HISTAMINE-CONTAINING NEURONS IN APLYSIA. D. Weinreich* (SPON: N. Brookes). Univ. Md. Sch. Med., Dept. Pharm., Baltimore, MD. 21201.

In the cerebral ganglia of Aplysia californica two identified neurons designated left and right C-2 are biochemically distinct because: 1) they contain at least 100-times more histamine (HA) than other neurons; 2) they synthesize and store ^{3}H-HA; and 3) they possess a 'specific' histidine decarboxylase enzyme. Intracellular stimulation of the C-2 neurons elicit postsynaptic potentials (PSPs) in several identified follower neurons. One set of followers receives a double inhibitory PSP consisting of a rapid IPSP (200 msec, 1-3mV) and a slow hyperpolarizing PSP lasting several sec. The amplitude and duration of the slow potential is greatly potentiated by repetitive stimulation and is abolished by lowering the temperature below 10° C. By contrast, the amplitude and duration of the rapid IPSP is enhanced by lowering temperature concomitant with a prolongation of the C-2 action potential (AP). Both PSPs are abolished by elevation of the extracellular Mg^{++} concentration, potentiated by increasing extracellular Ca^{++} concentration six-fold, and they have different reversal potentials. The rapid IPSP follows the presynaptic AP recorded in the C-2 somata one-for-one with a short and constant latency. Intracellular application of TEA ions prolongs the duration of the C-2 AP with a concomitant increase in the amplitude of both the rapid and slow PSPs. The somal membrane of these follower neurons is depolarized by acetylcholine and uniquely shows a hyperpolarizing response to iontophoretic application of HA; less than 5% of the 120 somata studied to date responded to exogenously applied HA. These results are consistent with the interpretation that some PSPs elicited by stimulation of the C-2 neurons are monosynaptic, chemical, and may be mediated by the release of HA. (Supported by NSF #BMS74-20270.)

1162 NEUROTRANSMITTER EFFECTS ON RNA SYNTHESIS IN CEREBELLAR CULTURES: A PROGRESS REPORT. Merrill K. Wolf, Mila Suva*, and Victor E. Shashoua. Dept. Anat., Univ. Mass. Med. Sch., Worcester, 01605, and McLean Hosp. and Harvard Med. Sch., Boston, 02215.

Treatment of mature organotypic cultures of mouse cerebellum with Bu_2-cAMP increased the uridine/cytidine (U/C) ratio in acutely synthesized RNAs. This indicates increased proportional synthesis of messenger and nuclear heterodisperse RNAs, and resembles the in vivo effects of Bu_2-cAMP or active learning. We have now used the cultures to test the effects of a variety of neurotransmitters on RNA synthesis. Mature, myelinated cultures were washed in balanced salts-glucose (BSS) for 4 hr, then incubated for 3 hr in 50 µl of BSS containing orotic acid-5-H^3 and the test compound, washed in BSS, and 3 to 6 pooled cultures homogenized in Tris buffer with 0.5% SDS. RNA was extracted and purified from the homogenates, hydrolyzed with KOH, the nucleotides separated by paper electrophoresis, and the radioactivity ratio of UMP to CMP corrected for the precursor pool and compared with controls incubated with orotic acid alone. The U/C ratio was increased 200% to 300% by Bu_2-cAMP, 3.2×10^{-4}M: 200% by noradrenalin, 8×10^{-5}M: 390% by GABA, 9.2×10^{-3}M: and 800% by acetylcholine, 9.2×10^{-3}M. Dose-response curves are being derived for noradrenalin and acetylcholine. cGMP, up to 3×10^{-2}M, and dopamine, up to 6.2×10^{-3}M, had no effect or lowered the U/C ratio. The increased U/C ratio appears to be produced by neurotransmitters endogenous to cerebellum but not by foreign ones. The increased U/C ratio was not obtained in cultures raised in a medium rich in nucleic acid precursors. (Supported by Grants #NS-11425 and NS-09407, NINCDS.)

1163 INTRACELLULAR INJECTIONS OF GABA AND GLUTAMATE INTO LOBSTER NEURONS. William R. Woodward* and Edward A. Kravitz (SPON: David H. Hubel). Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

We have investigated the transport of radioactively labeled transmitters in lobster neurons. Acetylcholine and serotonin, radioactively labeled by intracellular injection of precursors, are transported from cell bodies to axon terminals in invertebrate neurons (Goldman and Schwartz, J. Physiol. 242:61, 1974). The injection of transmitter substances and their precursors can be used to examine the specificity of transmitter transport (ibid.) and to identify transmitters in specific neurons. GABA is the transmitter used by inhibitory (I) neurons innervating exoskeletal muscles in the lobster, and glutamate has been suggested as the transmitter in excitatory (E) cells. Identified E and I cells in lobster abdominal ganglia have been injected with various ^{3}H-precursors and transmitters. Autoradiographs of nerve trunks at various distances from the cell bodies 48 hours after injection show that radioactive compounds are confined to the axons of injected cells. When GABA was injected into either E or I cell bodies approximately equal amounts of GABA moved out their axons (>30mm in 48 hours). If the nerves were ligated GABA accumulated on the proximal side of the ligature. When glutamate was injected into E and I cells, again roughly equivalent amounts of glutamate were found at distances of greater than 30mm in 48 hours. In addition, in I cells GABA was synthesized from injected glutamate, and the GABA:glutamate ratio increased distally in the axons. The results thus far show that glutamate and GABA are found out both E and I axons, suggesting a possible low specificity of transport of certain substances from cell body to terminals. Other test amino acids and amines will be injected into E and I cells to examine the specificity of the transport system and to differentiate active transport from passive diffusion of substances out the axons. (Supported by NIH).

Plasticity

1164 HIPPOCAMPAL FORMATION AS A MODEL SYSTEM FOR SYNAPTOGENESIS IN THE ADULT RAT. Anders Bjorklund* and Ulf Stenevi*. Dept. Histology, University of Lund, Lund, Sweden.

Immature central noradrenalin (NA), dopamine (DA) or serotonin neurons and immature or mature peripheral NA neurons survive transplantation to the hippocampal region in the adult rat: The transplants are placed in a cavity in the occipital cortex. During the subsequent months new fibers grow out from the transplanted neurons in rostral and rostro-lateral directions into the hippocampus, along a course approximately corresponding to that of the perforant path fibers. This fiber system is partly disrupted by the lesion, the hippocampus thus being partially deafferented in the transplanted animals. In some animals the brain stem adrenergic and serotonergic inputs were removed as well.

In this experimental situation the transplanted neurons will grow into the deafferented hippocampus and establish new terminal patterns above all in the dorsal part of the dentate gyrus and the CA3 region. The ingrowing DA and serotonin fibers terminated in a manner that closely mimicked that of the lesioned perforant path fibers, thus forming a dense terminal band in the outer portion of the molecular layer in the dentate gyrus. In contrast, the central and peripheral NA fibers ramified predominantly in the hilus of the dentate gyrus and close to the pyramidal cells in CA3; this corresponds to the distribution of the normal adrenergic innervation in the hippocampal region. In animals where an additional lesion was made in the hippocampal fimbria the terminal distribution of the ingrowing NA fibers expanded to cover also the basal part of the dentate molecular layer, i.e., into the region where septal cholinergic fibers normally terminate.

These findings demonstrate a remarkable ability of the central and peripheral monoaminergic neurons to establish highly reproducible and specific terminal patterns in the deafferented hippocampus. Moreover, in the reinnervation of the adult hippocampus the monoaminergic neurons seem able to replace or mimick other non-monoaminergic afferent fiber systems.

1165 SYNAPTIC REORGANIZATION OF GRACILIS AND CUNEATUS IN CAT AND MONKEY.
M.H. Feldman, F.W. Mis, and A.A. Sadun Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

The effects of chronic deafferentation of a major brainstem relay nucleus were examined by electrophysiological and anatomical methods in both cats and monkeys. The nucleus gracilis or cuneatus was deprived of its major synaptic input by excision of dorsal root ganglion or section of dorsal roots just proximal to the ganglion, from C-4 through T-2, or from L-3 through S-2, unilaterally. Following a recovery period of longer than 6 months, acute bilateral sensorimotor cortex lesions were carried out followed 4 or 5 days later by perfusion of the CNS for electron microscopy. Examination of the tissue by light microscopy showed a definite increase in the number of degenerating terminals in deafferented nucleus compared to the control side, using p-phenylenediamine (PPD) staining for degenerating terminals (Sadun, 1975). Electron microscopic examination of the nucleus cuneatus indicated a reorganization of synaptic inputs. A larger proportion of degenerating terminals was observed on the somata and proximal dendrites of neurons of deafferented nucleus than on the more distal portions of the dendritic tree.

Cats sustaining deafferentation of the nuclei gracilis and cuneatus via bilateral dorsal column C-4 transection, 3 to 10 weeks prior to acute experimentation showed marked differences in responsiveness of relay cells to both antidromic and orthodromic activation when compared to normal cats. Medial lemniscus (ML) stimulation with single shocks antidromically activated relay neurons by causing them to discharge in a time-locked repetitive burst. In control cats only a single time-locked response was noted. Orthodromic activation of these relay neurons by stimulation of the remaining upper cervical afferents also displayed a time-locked burst of repetitive firing of up to 7 or 8 spikes, in response to a single shock. In comparison, units in control cats responded with between two and three spikes.

Contralateral sensorimotor cortex stimulation does not normally activate relay cells but is directed at interneurons whose function is inhibitory to the relay cells (Andersen et al., 1964). In operated animals sensorimotor stimulation produced repetitive bursting in one quarter of the relay cells. No activation of relay neurons was seen in control cats following stimulation of the sensorimotor cortex.

Sensorimotor cortex stimulation normally modulates or inhibits orthodromic activation of relay cells, most probably via interneurons (Andersen et al., 1964). In operated cats when sensorimotor cortex stimulation precedes either ML or peripheral nerve stimulation it reduced the number of extracellular unit discharges per time-locked burst. This reduction of synaptically or antidromically activated bursting by the preceding cortical stimulus was dependent upon stimulus intensity, and frequently resulted in complete blocking of either or both anti- or orthodromic unit responses.

Intracellular studies of relay cells show evidence of postsynaptic membrane excitability changes. Multiple high frequency spikes were again observed following both synaptic and orthodromic stimulation with action potentials seen to arise during various times of the first spike's falling phase. After a short interval a third spike then arose from the baseline.

These findings suggest that synaptic remodeling of the primary sensory relay nuclei can occur following the removal of major afferents, probably as a result of the sprouting of presynaptic elements upon vacated postsynaptic sites. These modifications may account for the increased excitability seen in some neuronal systems following chronic deafferentation.

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1166 DIFFERENTIAL EFFECT OF STIMULATION OF THE PERFORANT AND COMMISSURAL PATH ON AXON TERMINALS AND DENDRITIC SPINES OF THE DENTATE MOLECULAR LAYER.

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The perforant path of the hippocampus yields postactivation potentiation, which is characterized by increased activity of the dentate granular cells to a single stimulus following brief tetanic stimulation of the path (Bliss and Lømo: J. Physiol, 232: 331, 1973). Swelling of dendritic spines of the dentate granular cells which make synaptic contacts with the stimulated pathway was suggested as a possible mechanism underlying the increased synaptic efficacy. Indeed such a swelling was demonstrated in regions of the dentate molecular layer which receive perforant fibers, whereas in the region occupied by commissural and septal afferents no swelling of dendritic spines could have been observed (Van Harreveld and Fifkova: Exp. Neurol, 49: 736, 1975). The poststimulation intervals studied ranged from 2-60 min. In electrophysiological experiments the potentiated response of granular cells outlasted the tetanic stimulus for number of hours even days (Bliss and Gardner-Medwin: J. Physiol, 232: 232, 1973; Douglas and Goddard: Brain Res, 86: 205, 1975). Presented experiments were aimed at investigating the morphology of spines and axon terminals during long periods of survival following stimulation of the entorhinal area. In 17 mice the entorhinal area was stimulated at 30/sec for 30 sec and animals survived the procedure for 4, 8 and 23 hrs. Controls (5) survived the sham procedure for 15-20 hrs. In the stimulated group in all survival intervals the dendritic spines were larger by 23% as compared to the controls. Axon terminals did not show any significant changes nor did the density of synaptic vesicles. Data of our previous experiments combined with those of the reported ones are summarized in the table. To test for the possible specific influence which the perforant path may have on synaptic morphology of the dentate molecular layer, in another group of 9 mice the commissural path, which projects to the proximal third of the dentate molecular layer, was stimulated (30/sec for 30 sec). The survival interval was 2-10 min. Unstimulated mice (9) with or without sham procedure were used as controls. The stimulated commissural path did not yield any changes in the synaptic morphology of either the proximal or distal third of the dentate molecular layer. This result points to specific properties of perforant fibers. Axon terminals of this path yield a transient change upon stimulation, which induces an endurable change in dendritic spines contacting these terminals. Swelling of dendritic spines may be triggered by glutamate released from axon terminals and axons similar to that described in other neural structures (Weinreich and Hammerschlag, Brain Res, 85: 137, 1975). Glutamate induced Na permeability of the spinal membrane could account for the initial but not for the later volume change of the spine. Here another mechanism, likely to involve increased protein synthesis may be accounted for. The long lasting volume increase of dendritic spines in the dentate molecular layer upon a short train of stimuli delivered to the perforant path supports the postulate which links such a change to the mechanism of long lasting postactivation potentiation observed in this pathway. (Supported by NIMH grant MH 27240)

Table: Percentile differences of mean values with standard errors between controls and stimulated mice (Controls taken as 100%)

Poststimulation interval	2-6 min	10-60 min	4-8 hrs	23 hrs
No of experiments	8	6	10	7
Spines	12.0 ± 3.4	38.6 ± 2.9	22.9 ± 8.1	24.1 ± 5.6
p	<0.01	<0.001	<0.02	<0.01
Axon terminals	-15.6 ± 4.2	3.7 ± 1.9	12.4 ± 5.6	14.7 ± 6.2
p	<0.01	NS	NS	NS
Vesicle density	-6.3 ± 4.3	-19.6 ± 7.1	0.8 ± 4.0	2.0 ± 4.3
p	NS	<0.05	NS	NS

1167 NORMAL AND ABNORMAL UNCROSSED RETINAL PROJECTIONS IN SYRIAN HAMSTERS AS DEMONSTRATED BY FINK-HEIMER AND AUTORADIOGRAPHIC TECHNIQUES.

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In normal hamsters the superior colliculus (SC) and dorsal and ventral nuclei of the lateral geniculate body (LGD & LGV) are innervated by both eyes. A point in the contralateral (cl) retina is represented along a line of projection, approximately orthogonal to the optic tract, extending inward from the external surfaces of SC, LGD, and LGV. Axons from the temporal retina which follow the ipsilateral (il) optic tract penetrate these structures along the lines of projection of corresponding points in the opposite retina, but terminate deep to levels receiving only cl retinal input. Thus, the two eyes innervate opposite ends of the lines of projection for the binocular field.

In adult animals with unilateral eye removal at birth (day 0) the il projection extends beyond its normal lines of projection in SC, but it remains confined to the rostral half tectum, as in normal animals. There is also an increase in uncrossed projections superficial to their normal major zone of termination at the upper border of stratum opticum. In LGV, the distribution of the il projection increases parallel to the optic-tract surface, as in SC; furthermore, the entire projection shifts superficially, thus leaving part of its normal zone of termination devoid of a direct retinal input. In LGD, where the zone deprived of cl input shows a 49% reduction in volume, the distribution of il optic input is not altered.

Adult animals with partial retinal lesions made on day 0 have an abnormal il projection to the region in SC, and the region in LGV, corresponding to part of the lesion; these il projections overlap partially with the zones of crossed innervation. The anomalous il axons in SC appear to consist of two populations: one is an increase in the normal projection, and is found only in the rostral tectum; the second shows the degeneration characteristics of the normal cl projection, and can be found superficially, even in the caudal tectum in cases of early lesions of the cl nasal retina.

We suggest that in LGV, and possibly in SC, uncrossed optic axons, like crossed retinotectal fibers, preferentially innervate unoccupied loci near the brain's surface. The distinctive distributions of the two populations in the abnormal il projection suggest that each is determined by the same factors as for the normal il and cl axons. In addition, the overlap of anomalous il projections with residual cl retinal input suggests that the same type of mechanisms govern the retinotopic distribution of cl and il terminals.

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1168 BIOCHEMICAL AND IMMUNOCYTOCHEMICAL EVIDENCES FOR COLLATERAL SPROUTING IN MESOLIMBIC DOPAMINERGIC NEURONS IN RAT BRAIN. G.M. Gilad, T.H. Joh, V.M. Pickel, and D.J. Reis. Lab. Neurobiol, Dept. Neurol., Cornell Univ. Med. Coll., New York, 10021.

We sought to establish in rats, if dopaminergic (DA) neurons of the mesolimbic system, projecting from mesencephalic cell bodies of the A10 group into the olfactory tubercle (OT), can: (a) undergo collateral sprouting in response to removal of non-DA input into OT; and (b) whether such growth of terminal axons is associated with changes in the activity of the catecholamine biosynthetic enzyme tyrosine hydroxylase (TH) within the cell bodies. A major non-DA input into one OT was removed by ablating the ipsilateral olfactory bulb. At various days thereafter the OT was examined for the development of collateral sprouting as evidenced by: (a) increased activity of TH; (b) increase in the high affinity uptake of ^{3}H-DA into crude synaptosome preparation of the OT; and (c) an increased number of immunocytochemical stained fibers containing TH in the OT. The A10 group was examined at the same time for changes in TH activity. In OT, TH activity began to increase by 10-14d, gradually reaching a maximum level of 125% of control ($p<0.005$) by 21d where it remained elevated for at least 60d. At the same time, dopamine-β-hydroxylase (DBH) activity did not change in the OT, furthermore, electrolytic lesions of the ascending noradrenergic projections at the level of the posterior midbrain resulted in a fall of DBH activity to 28% of control in the OT while leaving the elevated TH activity unaffected. Thus, the increase in TH activity in the OT was specifically associated with DA nerve terminals and not with the noradrenergic projections. By 30d the specific uptake of ^{3}H-DA into OT synaptosome preparation was increased to 121% of control ($p<0.05$). Immunocytochemical staining of TH by the peroxidase-antiperoxidase (PAP) method demonstrated a striking increase, by light microscopy, in the intensity of the staining within the OT, consequent to an increase in the number of stained fibers. In the DA cell bodies of the A10 group TH activity was transiently elevated to 121% of control ($p<0.05$) by 4d, returning to control level by 10d. We conclude: (a) that DA neurons of the mesolimbic system innervating the OT will sprout in response to removal of a major non-DA input and that the new innervation is sustained; (b) during collateral sprouting there is a transient elevation of enzyme activity in the uninjured A10 cell bodies which precedes the period of presumed growth; this elevation contrasts with the reversible reduction in TH during the retrograde reaction in injured A10 neurons which is associated with regenerative sprouting. The findings suggest that the increase in TH activity in the A10 cell bodies during collateral sprouting may be a reflection of an increase in the amount of enzyme protein required for transport into the enlarging terminal field. Thus, the biochemical mechanisms underlying collateral sprouting of uninjured neurons are not necessarily the same as those which are associated with regenerative sprouting of neurons in response to injury of their axons.

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1169 LACK OF COLLATERAL SPROUTING AND ITS PRESENCE AFTER SPINAL LESIONS IN ADULT CATS. M E. Goldberger and M. Murray, Dept. Anat., Med. Coll. of Pa., Phila., Penna. 19129

Collateral sprouting into cell nests of the nucleus gracilis is not seen to arise from a lumbar dorsal root which had been isolated by ganglionectomy of all the other ipsilateral lumbosacral and lower thoracic dorsal roots 18 months earlier. However, the spared root does show an increased projection to the caudal-basal portions of the nucleus gracilis. In contrast to the 'spared root preparation', a 'lowest remaining root preparation' was examined in which chronic lumbo-sacral-caudal deafferentation was followed 18 months later by T13 rhizotomy. The lowest remaining root projection is increased caudally in the base of nucleus gracilis and in the reticular portion above the obex. There is, again, little evidence of dorsal root sprouting among the cell nests despite the moderate increase in density of degeneration there. This increase may be due to shrinkage of that region. The total amount of sprouting from the lowest remaining root is less than that seen from the isolated, spared root. This is presumably due to the greater number of roots cut in the latter experiment. In fact, if the deafferentation is not extensive, sprouting may not be seen at all; either the amount of denervation is inadequate to elicit the sprouting response, or the collective sprouting of several remaining roots obscures and decreases the enlarged projection of only one of them. The spared root also increases its rostral projection to Clarke's nucleus (confirming Liu and Chambers, 1958) whereas the lowest remaining root does not. The intraspinal sprouting of the lowest remaining root is confined to the zona intermedia and ventral horn of its segment of entry plus the immediately adjacent segments.

Increased projection of descending systems occurs after complete lumbosacral deafferentation and is localized to certain parts of the gray matter. For example, sprouting was not seen in laminae II and III, or among the motor neurons of lamina IX. The increased descending projections were found from lamina IV and VIII and were most extensive in the zona intermedia. If however the deafferentation is incomplete, i.e. one root or fibers from several roots are spared, there is no sprouting from descending systems.

Thus, there is both negative and positive evidence for collateral sprouting after lesions of the adult spinal cord. Presence or absence of sprouting may be determined by several factors. 1) Incomplete lesions or too small a denervation may fail to elicit demonstrable sprouting either because the signal for sprouting is inadequate or because the sprouting is shared by a number of systems obscuring the single contribution of any one of the. 2) The sprouting response may be competitive and hierarchical among residual systems afferent to a partially denervated area; if one such system sprouts, it may pre-empt the available synaptic space. Thus both the quantity and pattern of sprouting depends not only upon the amount of denervation but also upon the population of remaining systems capable of sprouting. 3) The elicitation of sprouting may also be regulated by some undetermined relationship between the system denervated and the systems which remain. Regions in which afferent input is more strictly localized (cell nests of dorsal column nuclei, motor nuclei of spinal cord) do not show evidence of receiving collateral sproutins whereas regions of greater afferent overlap (reticular regions of dorsal column nuclei, zona intermedia) do.

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1170 EVIDENCE FOR REMODELLING IN LOCUS COERULEUS NEURONS FOLLOWING 6-HYDROXYDOPAMINE LESIONS. Jean Jew, Terence H. Williams, and Bang-Hsiung Hwang*. Dept. Anatomy, University of Iowa College of Medicine, Iowa City, Iowa 52242.

One of the weak links in the evidence for axonal growth in the mature mammalian CNS is the limitation in the resolving power of the light microscope. Through the years, many important observations of presumed regenerating (or growing) nerve fibers have been contributed by users of silver, cholinesterase, and catecholamine fluorescence techniques. These resolution limitations can be eliminated by the use of a glyoxylic acid catecholamine fluorescence method that permits viewing of the same or adjacent sections by EM (Chiba and Williams, Histochemistry, in press).

The first perfusate is cold 2%(w/v) glyoxylic acid in Krebs-Ringer bicarbonate buffer at pH 7.0. The second contains 0.5% glutaraldehyde and 4% paraformaldehyde in Sorensen's phosphate buffer at pH 7.3. Vibratome sections of 30μ and 60μ thickness are given a second exposure to glyoxylic acid (2% glyoxylic acid in phosphate buffer, pH 7.0, for 5 to 10 minutes), and mounted in 50% glycerin-water. When fluorescent elements of interest are identified, the sections are rinsed in 7% sucrose in water and processed for EM.

Using this protocol, changes have been studied after 6-hydroxydopamine-induced lesions placed by stereotaxis in the locus coeruleus of 150 gram rats. Four to six days after the lesion, fluorescence microscopy revealed thickened varicose reactive fluorescent processes; also numerous rounded intensely fluorescent bodies among the noradrenergic neurons in the lesioned area. The latter resembled, in size and brilliance, SIF cells of sympathetic ganglia. EM correlations using the same and adjacent sections disclosed many conspicuous axonal expansions. Many of these contained tubular elements, filaments, mitochondria, and pleomorphic vesicles, such as have been described in growth cones in tissue culture (Bunge, 1973, J. Cell Biol). The fate of these presumed "growing" axons is being studied after 2, 4, and 8 week survival periods. Supported by NS-11650-02.

1171 REVERSIBLE REDUCTION IN TYROSINE HYDROXYLASE ENZYME PROTEIN DURING THE RETROGRADE REACTION IN MESOLIMBIC DOPAMINERGIC NEURONS. D.J. Reis, G.M. Gilad, V.M. Pickel and T.H. Joh. Lab. Neurobiol., Dept. Neurol., Cornell Univ. Med. Coll., New York, 10021.

Previous studies from this laboratory have demonstrated in rat that the retrograde reaction initiated in central noradrenergic neurons in the locus coeruleus (Ross et al., Brain Res., 92:57, 1975) and dopaminergic (DA) neurons of the nigro-striatal system (Reis et al., Neuroscience Abstracts, 1:500, 1975) by distal axon lesions is characterized by a reversible reduction in the activity and amount of tyrosine hydroxylase (TH), the enzyme catalyzing the first step in catecholamine biosynthesis. The present study sought to determine if comparable changes in TH occur in central DA neurons of the mesolimbic system which project from mesencephalic cell bodies of the A10-DA group into the olfactory tubercle (OT). Axons of A10 neurons were transected ipsilaterally by electrolytic lesions either close to the cell bodies in lateral hypothalamus, or near the terminal axons in the OT. At various days thereafter the animals were killed and the A10 area and the OT removed to determine TH activity and, by immunotitration with a specific antibody to TH, the relative amount of the enzyme. In other animals DA neurons in the midbrain were examined immunocytochemically by staining them with a specific antibody to TH using the peroxidase-anti-peroxidase (PAP) method. Hypothalamic lesions resulted in a rapid and permanent fall in the activity and amount of TH in the OT to 10-20% of control ($p<0.001$). In the A10 neurons TH activity first increased within 24h to 133% ($p<0.01$) and then declined to reach 40% of control by 14d. The permanent decrease in TH activity was due to the immunocytochemically demonstrable loss of DA neurons. In contrast, lesions in the OT resulted in a reversible decline in the activity and amount of TH in the A10 neurons reaching 70% of control ($p<0.01$) by 7d, remaining depressed for a week, with recovery by 21d. No cell loss was evident. We conclude that a reversible reduced accumulation of TH characterizes the retrograde reaction in DA neurons of the mesolimbic system, as in the nigro-striatal and noradrenergic systems of the locus coeruleus in response to lesions of distal axons, and that the rapid anterograde decline in TH is similar in both DA systems differing from the slow decline in central noradrenergic neurons. A reversible reduction in the enzymes synthesizing neurotransmitters may be a hallmark of the retrograde reaction in CNS neurons and may reflect a reordering of protein biosynthetic pathways in regeneration, favoring accumulation of proteins required for reconstitution of cell membranes at the expense of those used in neurotransmission.

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1172 AN AUTORADIOGRAPHIC ANALYSIS OF IPSILATERAL RETINAL PROJECTIONS OF THE INTACT OR REGENERATED OPTIC NERVE OF RANA PIPIENS AND THE EFFECT OF ENUCLEATION OF THE OPPOSITE EYE. Dennis J. Stelzner. Dept. of Anatomy, Upstate Medical Center of SUNY, Syracuse, New York, 13210.

(^{3}H) proline (5-80 uCi) was injected intraocularly in normal frogs which survived for 18-24 hrs. (n=6) or seven days (n=4) before being prepared for autoradiography. Contralateral retinal projections were similar to those described in previous studies. In all animals, ipsilateral retinal projections were found that have not been previously reported. A small but definite increase in the number of silver grains above background was found in the ipsi basal optic nucleus (BON). Equally surprising was a direct projection to the rostral pole and lateral wall of the optic tectum (OT) limited to a band in the superficial third of the stratum griseum centrale. The ipsi projection to the neuropil of Bellonci (NB) did not fill lateral and medial portions of the plexus or the ventral half of the core. In the anterior portion of the ipsi corpus geniculatum thalamicum (CGT) patches of label were found in a dorsomedial area of the neuropil and in a zone along the base. More posteriorly in CGT, the ipsi projection was restricted to the medial edge of the neuropil. These ipsi projections to the anterior thalamus overlapped the contra projections.

In 5 frogs the right optic nerve was crushed and allowed to regenerate. Normal visual striking behavior recovered after 1-2 mo. After 6 mo. the right eye was injected with (^{3}H) proline (40 uCi). In all 5 frogs there was an increased amount of label above normal in the ipsi optic tract and an anomalous projection to the superficial layers of ipsi OT which was lower in density than the normal contra projection. There also was an apparent increase in the amount of label in BON. Although the basic pattern of label in the anterior thalamus was similar to normal, little label was found in the dorsomedial sector of the anterior part of CGT or in the core of NB. Also, the label in the posterior part of CGT was not as restricted to the medial edge of the neuropil.

In 6 frogs the right optic nerve was crushed either at the same time the left eye was removed (N=4) or 3 months after it was removed (N=2). After 6 mo. the right eye was injected with (^{3}H) proline. In all frogs there was an increased amount of label in the ipsi optic tract and ipsi OT when compared with normal or regenerate animals. There was also an obvious increase in label in ipsi BON. Little label was found in the dorsomedial portion of the anterior part of CGT or in the core of NB but label filled the rest of the neuropil in these regions.

In 9 frogs the left eye was enucleated and the right eye was injected with (^{3}H) proline either 1 mo. (N=2), 3 mo. (N=2) or 6 mo. (N=5) later. The autoradiographic results described below were noticeable at 1 mo. but were not obvious until 6 mo. survival. The ipsi NB, CGT, and OT were obviously shrunken after being chronically deafferented of contra retinal input. In spite of this shrinkage of the neuropil, label spread to fill the entire CGT and most of the NB. There was also a spread of label across the tectal midline for 200-250 um which filled the superficial layers of the ipsi OT. The normal band of ipsi label found in the lateral wall of OT was increased in density and spread dorsomedially. There also was an apparent increase in label in the ipsi BON.

These findings are most easily interpreted to indicate that the factors which limit growth of axons into the ipsi optic tract are no longer operative during regeneration. Moreover, even in the post-metamorphic frog, there is an interaction between ipsi and contra retinal axons for sites in the thalamus and midbrain. When contra axons are removed, intact or regenerated retinal axons grow into regions formerly excluded them by the contra retinal projection.

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1173 INDUCTION OF THE FIELD COMPRESSION WITHIN A ROTATED TECTAL REIMPLANT IN GOLDFISH.

Myong G. Yoon, Dept. of Psychology, Dalhousie University Halifax, Nova Scotia, Canada.

The visual pathways in adult goldfish are capable of readjusting to various types of experimentally induced size-disparity between the retina and the optic tectum in a consistent topographic order. Following excision of the caudal half of the tectum, the remaining rostral half-tectum eventually acquires visual projections not only from the appropriate temporal area of the retina but also from the foreign nasal half of the retina in an orderly compressed topographic pattern. On the other hand, if a piece of the tectal tissue is dissected out, lifted free, and then reimplanted to the same tectum in adult goldfish, the re-implanted tectal tissue retains its original topographic polarity regardless of the orientation of reimplantation after either a rotation or even after an inversion of its entire laminar structures.

The present work shows that it is possible to induce a field compression onto a rotated tectal reimplant within the halved tectum in adult goldfish as follows: a piece of the tectal tissue was dissected from the central area of the whole tectum, and then reimplanted after either 180 or 90 degrees rotation. When the reimplanted tectal tissue became reinnervated by optic fibers later, the caudal half of the operated tecum (including the posterior half of the reimplant) was excised. The remaining rostral half of the operated tectum acquired visual projections from the whole extent of the visual field. Furthermore, the re-established visual projections onto the reamining part of the halved tectal reimplant within the rostral half-tectum showed a field compression in accordance with the original topographic polarity of the 180 or 90 degrees rotated tectal tissue. In a further study, a field compression was induced first in the intact rostral half-tectum following excision of the caudal half, and then a piece of the 90 degrees rotated tectal tissue was reimplanted later within the rostral half-tectum. The field compression in the re-established visual projections onto the reimplanted area showed a corresponding localized 90 degrees rotation. These results suggest that the retention of original topographic polarity by a reimplanted tectal tissue is compatible with the capability of the same tectal tissue in readjusting to a size-disparity in adult goldfish.

Histological examination of the operated half-tectum with a reimplant (stained by a modified Golgi method) revealed that the reimplanted tectal tissue retained highly organized cytoarchitectonic structures. Various types of tectal neurones maintained intricate interconnections within the reimplanted tissue. These neuronal elements of the reimplanted tectal tissue may be responsible for the retention of original topographic polarity of the tectal tissue, and also for its dynamic capability in readjusting to a size-disparity.

(Supported by grants from MRC and NRC of Canada)

1174 INFANT RATS: THE ONTOGENY OF SENSORIMOTOR BEHAVIORS AND THE EFFECTS OF DESTRUCTION OF THE NIGROSTRIATAL BUNDLE. C. Robert Almli and Robin S. Fisher*. Dept. Psychol., Ohio Univ., Athens, Ohio 45701.

Male and female infant rats (ten or 25 days of age) received Bilateral or Unilateral damage of the substantia nigra-nigrostriatal bundle (NSB). The sensorimotor deficits and symptoms displayed by the brain-damaged infants was compared to normal ontogeny of sensorimotor capacity, and the brain-damage rats were tested through development for feeding-drinking regulatory capacity. The Bilateral-NSB infant rats showed aphagia-adipsia, body weight loss, and sensorimotor disturbance immediately post-lesion. The sensorimotor deficits were transient; and included abnormality of righting, hopping, placing, and grasping responses; as well as deficits in visual, auditory, olfactory, and somatic sensory orientation. Motor symptoms included postural and gait abnormatity, spasticity, tremor, and obstinate progression. The sensorimotor abnormalities tended to be bilateral for the Bilateral-NSB group. The deficits of the Unilateral-NSB rats were typically of shorter duration, and the deficits tended to be ipsilateral to the lesion site. The Bilateral-NSB rats displayed permanent deficits in responding to a variety of feeding-drinking challenges, while the Unilateral-NSB rats showed minimal regulatory deficits. Thus, similar to studies conducted with adult rats, damage to the NSB of pre- and post- weanling rats yields a syndrome of regulatory and sensorimotor abnormality strickingly similar that that produced with lateral hypothalamic area destruction.

1175 LATERAL GENICULATE CELLS WITH SIMILAR CONDUCTION TIMES CLUSTER: A NEW ORGANIZATIONAL PRINCIPLE IN THE CAT? C. W. Ballard* and W. L. Salinger (SPON: M. R. Harter). Depts. Math, Psych. Univ. of N. C. Greensboro, NC 27412.

Students of the lateral geniculate nucleus (LGN) have suggested that cells of the same functional type (X or Y) seem to lie in groups. Statistical analyses of the effects of monocular paralysis on the LGN of the cat indicate that, in acutely monocularly paralyzed (MP) cats, whose LGN's appear essentially normal, cells characterized by response latencies from optic chiasm shock (OX latency) above and below 1.6 msec. do indeed cluster. Having found a cell on one side of a point of dichotomization of the latency values, it is highly probable that the next cell will lie on the same side of that division point. Only dichotomization in the range of 1.6 - 2.0 msec. (the presumed boundary region separating X- and Y- cells) yields an indication of highly significant grouping. Because chronic monocular paralysis apparently alters both the morphology and physiology of the LGN, it seems reasonable that clustering may be altered as well. LGN cells of the chronically MP cat are grouped tightly. Moreover, this clustering is significant with points of dichotomization ranging from 1.2 to 2.3 msec: a situation arising, in all likelihood, from the tight clustering of cells with similar latencies. These data suggest, in addition to organization based on X or Y characteristics, a further organizational scheme based on morphological properties reflected by different afferent conduction velocities. Cells with similar OX latencies, therefore, cluster tightly, a grouping coextensive with clustering of X- and Y- cells. The comparison of acute and chronically MP animals suggests that this organizational pattern is present in acutely paralyzed (and, presumably, normal) cats but becomes accentuated by changes induced by chronic monocular paralysis.

1176 AXONAL REGENERATION IN IMMATURE AND ADULT RATS, Black, M.M.*and Lasek, R.J., Dept. of Anatomy, Case Western Reserve Univ., Cleveland, Ohio.

The accurate determination of the rate of axonal elongation is important because this rate can be correlated with the rate of transport of axonal structures. These correlations lead to testable hypotheses concerning the role of these structures in axonal elongation. We have used peripheral nerve regeneration as a model for axonal elongation. The system is the ventral motor neuron of the rat sciatic nerve. Regeneration was assayed by axonal transport of labelled materials to the growing tips of regenerating axons. A ^{3}H-lysine-proline mixture was injected bilaterally in the ventral gray at spinal cord levels L5 and L6. Regeneration was initiated immediately afterwards by crushing each sciatic nerve for 5-7 sec with a hemostat. The distribution of radioactivity along each sciatic nerve was determined in rats sacrificed 4 or 8 days after crushing the nerve. The distance regenerated was defined as the region between the crush site and the front of radioactivity. In adult rats the distance regenerated was 4.9±1.4mm (means±s.d, n=9) and 19.3±2.9mm (n=7) after 4 and 8 days respectively. The rate of regeneration from 0-4 days was 1.2mm/day, while from 4-8 days it was 3.6mm/day. This difference indicates a delay exists early in the regenerative process. This delay could result from mechanical obstruction at the crush site and/or a latent period during which the axons are readied for elongation. The distance regenerated was also determined 4,7 and 8 days after crushing the nerve in immature rats (3 wks old). The axons regenerated 8.6±3mm (n=8), 22.5± 1.7mm (n=4) and 27.8±3.8mm (n=4) respectively. The distance regenerated in pups was statistically greater than in adults ($p<.01$). Possible explanations for this include a faster regeneration rate and shorter delay in pups. These observations will be discussed in light of our current understanding of axonal transport.

1177 A DETERMINATION OF THE INTERVAL BETWEEN EARLY LESIONS OF THE SUPERIOR COLLICULUS AND THE APPEARANCE OF AN ANOMALOUS RETINAL PROJECTION IN THE SYRIAN HAMSTER. Barbara J. Crain and Michael A. Owens*, Departments of Anatomy and Psychology, Duke University, Durham, North Carolina 27710.

The superficial layers of the hamster superior colliculus were ablated unilaterally at birth. Four to 10 days later the animals received bilateral enucleations. After 24 hours the animals were sacrificed and their brains were processed according to the Fink-Heimer technique. Four days after the tectal lesion, no differences were detected between the experimental and control sides in either the amount or the location of degeneration. By 6 days, the retinal projection to the experimental side had expanded into the lateral posterior nucleus (LP), the ventral lateral geniculate, and the remaining deep layers of the superior colliculus. However, these anomalous projections were not nearly as prominent as they are in the adult (Schneider, Brain, Behav. Evol., 3: 295, 1970). Animals which received enucleations 8 or 10 days after their tectal lesions showed degeneration patterns which were indistinguishable from those of the adult. The presence of optic tract terminals in LP 6 days after removal of the superior colliculus was verified electron microscopically. At this age, growth cones were still present. Fewer synapses were observed than in the adult, and most of these had relatively few, scattered synaptic vesicles. None of the synaptic complexes characteristic of the adult LP was seen, nor was there any evidence of myelination. The finding of anomalous retinal terminals in LP only 6 days after tectal ablation will be useful in understanding the differences between the normal synaptic ultrastructure of the mature LP and the synaptic organization found in the adult after neonatal tectal lesions. Supported by NINDS Grant #NS-09623 to W. C. Hall.

1178 GOLGI ANALYSIS OF KINDLED HIPPOCAMPAL FOCI OF RATS. James E. Crandall,* Jerald J. Bernstein, Carl A. Boast* and Steven F. Zornetzer. (SPON: Frederick A. King). Dept. Neuroscience, Coll. Med., Univ. Fla., Gainesville, Florida 32610

Long lasting changes in neural activity (the kindling effect) result from daily, repeated low level stimulation of the brain. Morphological evaluation of kindled amygdaloid or cortical foci suggest no obvious histological alteration in the tissue surrounding the stimulation site. In contrast, chemically-induced or naturally occuring epileptic foci result in a number of reported neuroanatomical changes (spine loss, dendritic nodulation, decreased dendritic field, etc.). Recently, morphological alterations resulting from electrical brain stimulation have been reported. If kindling represents a model for induced neuronal plasticity, morphological changes in altered tissue might be expected. The present study investigated possible neuroanatomical correlates of kindling in the hippocampal formation of rats. Bipolar electrodes were bilaterally implanted into the ventral hippocampal region. Two groups of animals were evaluated: a group receiving daily stimulation and a group of implanted nonstimulated control rats. All experimental rats were stimulated unilaterally and electrographic as well as behavioral measures were made. Following a determined behavioral endpoint, rats were sacrificed and brains processed using a Golgi-Cox modification. Qualitative and quantitative measures from coded tissues were used to evaluate several morphological parameters (dendritic varicosities, extent of dendritic field and degree of dendritic branching) of the tissue surrounding the electrode tip. Results will be discussed in terms of morphological substrates of experimentally induced altered neural activity.

1179 LONG LASTING PERFORANT PATH POTENTIATION WITH VERY BRIEF TETANIC BURSTS. Robert M. Douglas* (SPON: G.V. Goddard), Dalhousie Univ., Dept. Psychol., Halifax, Nova Scotia, Canada

Monosynaptic field potentials were recorded in the fascia dentata of the rat hippocampus in response to perforant path stimulation. In unanaesthetized rats a long lasting potentiation (>24 hrs) of both the population EPSP and spike components of the evoked response was induced by a series of very brief trains (2-10 pulses) presented at a very low repetition rate of one every two to ten seconds. The pulses within each burst were separated by 2.5 to 5 msec. Besides roughly mimicing the complex spike configuration often observed in single unit recordings, the procedure had other advantages. No afterdischarge was observed. Rather, the response to each burst was very similar to that evoked by single test pulses, with only one population spike being emitted by the post synaptic elements, the granule cells. Furthermore, the potentiation was still obtained if this one spike was blocked by recurrent inhibition generated by a prior perforant path input to the granule cells. The results suggest that the potentiation may be produced by normal brain processes, and that either the presence or timing of the post synaptic discharge is not critical.

1180 THE EFFECT OF MONOCULAR DEPRIVATION ON MOUSE STRIATE CORTEX. Ursula C. Dräger, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

In the binocular region of mouse primary visual cortex more than 2/3 of all cells respond to stimulation through both eyes, the contralateral eye providing the overall dominant influence. It was possible to produce marked changes in this normal ocular dominance distribution by early monocular deprivation: in the hemisphere contralateral to the deprived eye the majority of cells were still binocular, but the ipsilateral (experienced) eye was now stronger; in the cortex ipsilateral to the deprived eye almost all cells responded exclusively to the contralateral (experienced) eye. The projection to the striate cortex can be made visible anatomically by transneuronal autoradiography after injecting one eye with radioactive tracers. With this method one expected to see an unusually strong ipsilateral geniculo-cortical projection after injection of the experienced eye, and no ipsilateral projection after injection of the deprived eye. Instead, the autoradiographs showed a roughly normal ipsilateral projection regardless of the eye injected. One interpretation of these conflicting physiological and anatomical results could be that through monocular deprivation the ipsilateral projection becomes in some way non-functional, but does not disappear. This suggestion was to some extent support by repeating in the mouse the experiment of Kratz et al. (1), of enucleating the experienced eye: after eye removal, the deprived eye's influence on cells in the ipsilateral hemisphere did not change immediately (contrary to the results in the cat), but within 3-5 days almost all cells in the upper cortical layers became responsive to visual stimulation, although the responses seemed on the whole pathologic. Cells in the deeper parts of the cortex remained unresponsive, even 4 weeks or more after enucleation.

(1) Kratz, K.E., Spear, P.D. and Smith, D.C., Post-Critical-Period Reversal of Effects of Monocular Deprivation on Striate Cortex Cells in the Cat. J. Neurophysiol. 39 (1976).

1181 PHOSPHODIESTERASE INHIBITORS MODIFY SYNAPTIC ACTIVITY IN THE HIPPOCAMPUS. Thomas V. Dunwiddie*, Valentin K. Gribkoff* and Gary S. Lynch. Dept. of Psychobiology, UCI, Irvine, CA. 92717

A parametric investigation was made of the factors involved in post-tetanic potentiation of the monosynaptic Schaffer collateral response of the hippocampal slice preparation. Several xanthine-derivative and non-xanthine phosphodiesterase inhibitors were found to cause changes in the normal response, which were in many respects similar to those found after tetanic stimulation of this pathway. A ten minute treatment in media with PDE inhibitor was found to produce prolonged increases in the sensitivity of this pathway, and appeared to have an interactive effect with the potentiation phenomenon. This evidence suggests a role for cyclic nucleotides in modifying the characteristics of this highly labile hippocampal synapse.

1182 EFFECTS OF AXOTOMY ON THE MONOSYNAPTIC RESPONSE OF MOTONEURONS TO LATERAL COLUMN STIMULATION IN FROG. Paul B. Farel, Gábor Czéh*, and James N. Weakly. Dept. Physiol., Sch. Med., Univ. N. Car., Chapel Hill, N.C. 27514

The ninth ventral root (VR9) of Rana pipiens was sectioned on one side. After periods of 7-37 days, the spinal cords were removed, placed in oxygenated glucose-Ringer's solution, and prepared for electrophysiological recording of ventral root reflexes from VR9 and VR10 elicited by lateral column stimulation (LC-VRR).

Terminals of LC fibers are located primarily on soma and proximal dendrites of motoneurons. In cat, ventral rhizotomy produces preferential loss of somatic synapses. The mean ratios (VR9/VR10) of reflex parameters shown below support a similar conclusion in frog (n = 6 for each group).

	AMPLITUDE LC-VRR 9/10	REFLEX LATENCY 9/10
UNOPERATED	1.39	.84
7-11 DAYS POSTOP.	.41*	.87
23-37 DAYS POSTOP.	.26*	1.45*

* indicates statistically significant difference from unoperated group (2P = .05, t-test).

Note that the decrease in amplitude of the LC-VRR recorded from the sectioned ventral root (VR9) precedes the increase in reflex latency. Data derived by recording focal potentials within the motoneuron pool indicate that LC conduction velocity and synaptic delay are unchanged. Therefore, the conduction velocity of the severed VR9 fibers must have decreased.

Preliminary intracellular studies suggest the presence of dendritic spikes in axotomized motoneurons, but not in motoneurons of adjacent intact segments.

1183 ENHANCED RECOVERY OF HINDLIMB REFLEX ACTIVITY IN RATS WITH SCIATIC NERVE CRUSH BY POST-OPERATIVE POLYAMINE ADMINISTRATION. Kevin R. Fox* and Warren C. Stern (SPON: R. Schoenfeld). Squibb Inst. Med. Res., Princeton, N. J. 08540.

Polyamines, notably spermidine and spermine, are endogenously synthesized in the peripheral and central nervous systems. There is a strong positive correlation between the concentration of these amines and nucleic acid activity, protein synthesis and tissue growth. Since these processes are likely to be involved in recovery from nerve damage we assessed whether administration of polyamines following injury to the sciatic nerve would enhance recovery of reflex activity. The return of hindlimb reflexes after unilateral hemostat-induced sciatic nerve crush was determined in adult rats given daily post-injury injections of spermidine trihydrochloride (10 mg/kg, n = 15), spermine tetrahydrochloride (10 mg/kg, n = 15) or saline (n = 13). Reflex measurement, conducted blind, consisted of exposing the paw of the injured limb to a hot punctate light source and recording the latency for limb withdrawal. Both polyamine treated groups showed faster recovery rates than the saline rats with the spermine group having significantly shorter reflex latencies on post-injury days 2 and 3. Polyamine injections did not alter reflex latencies of the non-injured hindlimb. All groups returned to pre-injury baseline latencies by 9 days following nerve crush. It appears that administration of polyamines following peripheral nerve damage is a promising new approach to the pharmacological enhancement of recovery.

1184 ENVIRONMENTAL COMPLEXITY VERSUS ISOLATION: A SENSITIVE PERIOD FOR EFFECTS ON CORTICAL AND HIPPOCAMPAL DENDRITIC BRANCHING IN RATS? William T. Greenough, Frederick M. Snow* and Beverly A. Fiala*. Dept. of Psychol. & Prog. Neural & Behav. Biol., Univ. Illinois, Urbana-Champaign, IL. 61820

Behavioral studies by others suggest effects of environmental complexity (EC) and individual cage (IC) housing depend on age. Studies of cortical weight and thickness by others, in contrast, indicate effects of exposure at any age. We previously reported greater dendritic branching of EC occipital cortical neurons after 30 days postweaning exposure (Greenough & Volkmar, Exp. Neurol. 40:491,1973). We have seen postweaning effects to varying degrees across 3 independent replications. We now describe 2 replications in which male littermate pairs of 90 day old Long Evans hooded rats were housed in groups with a daily set of toys and exploration period (EC) or alone in standard cages (IC) for 90 days. The first replication (N=6 pairs) used 100 micron posterior cortical sections stained by the Ramon-Moliner (Stain Technol.32:105,1957) Golgi technique. The second replication (N=4 pairs) used Golgi-Cox stained sections. In each replication approximately 15 Layer 4 occipital cortex pyramidal and 15 stellate neurons were drawn, at 500X with a camera lucida, from each subject and scored for number and length of dendritic branches and the number of intersections of branches and an overlay of concentric rings at 20 micron intervals. Results indicated no consistent pattern of effects of differential housing. We conclude that effects of differential housing on adult dendritic branching are very small or non-existent, although we have seen small effects of extensive maze training in adults. In a separate study, dendritic branching in hippocampal dentate granule cells of rats exposed for 30 days postweaning differed by about 25% by ring intersection analysis. Adult hippocampal studies are in progress.
--Supported by NICH&HD grant HD 06862 and NSF BMS 08596.

1185 HABITUATION-LIKE DECREMENTS IN TRANSMISSION THROUGH A NORMAL AND A LESION INDUCED PATHWAY IN THE HIPPOCAMPAL FORMATION OF THE RAT. E.W. Harris* and O. Steward. (SPON: D.H. Cohen). Depts. of Neurosurg. and Physiol. Univ. of Va. Sch. Med., Charlottesville, Va. 22901.

Destruction of the projection from the entorhinal cortex (EC) to the dentate gyrus of the rat results in the reinnervation of the partially deafferented dentate by fibers from the contralateral EC. As these share many anatomical properties with the normal ipsilateral EC projections, it was of interest to determine whether the lesion-induced projections also behaved physiologically like the normal ipsilateral circuit. An approach to this question is to examine the physiological properties which characterize the system, and attempt to describe a distinctive physiological signature for the normal pathway. The lesion-induced crossed pathway may then be analyzed in terms of the features of this normal signature. Stimulation of the EC results in monosynaptic activation of the dentate granule cells which may be recorded extracellularly as a synaptic wave and a population spike reflecting granule cell discharge. One feature of this pathway is a response decrement resembling habituation, observed when stimulating at frequencies of 1/15sec.-1/sec. All 8 of the applicable parametric criteria used to define habituation (Thompson and Spencer, Physiol. Rev. 73, 16-43, 1966) have been met for the normal projection in vitro (Tyler and Alger, Brain Res., in press) and have been observed in the present in vivo studies. Investigation of the lesion-induced crossed pathway with an habituation paradigm revealed response decrements having a time course comparable to that observed when stimulating the normal ipsilateral circuit at similar frequencies. This habituation-like phenomenon in the lesion-induced crossed projection will be compared with that of the normal ipsilateral pathway with respect to the 8 applicable criteria for habituation. (Supported by USPHS Research Grant No. 1 R01 NS12333 to O. Steward).

1186 THE FORMATION OF AFFERENT ANATOMICAL CONNECTIONS WITH HETEROTOPICALLY TRANSPLANTED CEREBELLAR TISSUE IN THE RAT: A FINK-HEIMER STUDY. Rodney J. Hine* (SPON: Joseph Altman). Dept. of Biological Sciences, Purdue University, W. Lafayette, IN 47907.

Cerebellar anlage from 18-day-old rat fetuses were transplanted into the rostral forebrain of 7-day-old neonate rat hosts. At 4 to 6 months of age, lesions were made in various telencephalic and diencephalic sites of the host brains. The hosts were sacrificed 8 days after the lesion, and the brains were processed according to the Fink-Heimer method. The transplanted cerebellar cortex exhibited the characteristic trilaminar pattern of organotypic differentiation. In several cases degenerating fibers and terminals could be seen within the transplanted tissue. Degenerating fibers typically coursed through a medullary layer before passing into the internal granular layer of the transplant. Degeneration was most extensive when the transplant was located near or within the normal projection area of the ablated tissue or along its efferent pathways.

These light microscopic findings indicate that the developing mammalian CNS is capable of forming lasting anatomical connections with the neurons of heterotopically transplanted fetal CNS tissue. These afferents may represent newly-formed collaterals of immature pre-existing axons which either arise in response to or are maintained by the presence of the deafferented cerebellar transplant. (Author supported by NIH Neurobiology Training Grant MH10267-11; Research conducted in the laboratory of Dr. G. D. Das under NIH grant NS08817-05)

1187 EVIDENCE FOR AXONAL REMODELLING IN A CENTRAL NORADRENERGIC TRACT FOLLOWING ELECTROLYTIC LESION. Bang-Hsiung Hwang*, Tanemichi Chiba, Asa C. Black, Jr., and Terence H. Williams. Dept. Anatomy, University of Iowa College of Medicine, Iowa City, Iowa 52242.

Axonal remodelling was examined after electrolytic lesions involving the medial forebrain bundle at the caudal hypothalamus, using a combined glyoxylic acid fluorescence and electron microscope technique developed in our laboratories (Chiba, et al., 1976, Histochemistry, in press). A fine network of varicose fluorescent fibers was observed in the proximal portion of the lesion site 2 to 4 weeks after operation. In the areas that corresponded to the distribution of these varicose fluorescent fibers, presumed "growing" axon profiles were observed. These contained pleomorphic vesicles, dense-cored vesicles, smooth-surfaced tubules, dense bodies, and some mitochondria. Presumed "growing" axons were found 4 days and 2, 4, and 8 weeks after lesion. They were most frequent 2 weeks after lesion in the dorsocaudal portion of the lesion site. Most of these presumed "growing" axons are believed to have originated from noradrenergic axons in the dorsal tegmental bundles, since they were confined to the zone where reactive (varicose) fluorescent fibers were identified. Perivascular intense fluorescence observed 8 weeks after lesion was proved by electronmicroscopy to be hyperinnervation of blood vessels. Some examples of presumed "growing" axonal profiles were seen in close relationship to the endothelial cells of small blood vessels. Supported by NS-11650-02.

1188 SYNAPTIC CONNECTIONS MUST CHANGE IN THE GROWING ADULT GOLDFISH RETINA. Pamela R. Johns. Div. Biol. Sci., Univ. Michigan, Ann Arbor, MI 48109.

Retinal growth in adult goldfish (1-2 yrs. of age; 5-12 cm body length) was studied radioautographically following intraocular or intraperitoneal injections of ^{3}H-thymidine into 5 cm fish. With short survival times (12 hrs.-4 days) labeled retinal cells are found exclusively around the margin, at the ora terminalis (OT). With longer survival times (28-336 days) the annulus of labeled cells is progressively displaced from the OT by new cells added subsequently. The amount of new retina added dorsally is greater than that added ventrally by about 20% at 336 days; nasal is greater than temporal by slightly less. The retina also grows by hypertrophy; the retinal region central to the labeled annulus has doubled in size by 336 days. This stretching is reflected in a decrease in the density (#/mm^2 retinal surface) of all cells except the rods, whose density remains constant. What is the source of these extra rods? Labeled rod nuclei are not confined to the annulus formed by the other labeled cells; they spread centrally, but not all the way to the posterior pole. A region of central retina about the size of the entire retina of a 5 cm fish contains no labeled rods.

I interpret these results to mean that the retinal growth has two components: (1) concentric rings of new cells are added at the OT, and (2) previously formed retina increases in size by expansion of all cell layers except the rods, which remain relatively fixed in position in the central retina. The gap thus created in the rod layer at the periphery is filled by new rods migrating in from the OT. This shearing between the rod layer and the rest of the retina means that a given rod will be adjacent to different bipolar and horizontal cells at different times. Since long laterally directed rod axons have never been reported, the rods must be constantly changing their synaptic connections. Supported by EY-00168 & MPMP 494 to S. Easter; MH051185 & Fight for Sight Student Fellowship to PRJ.

1189 VESTIBULO-OCULAR REFLEX (VOR) IN CATS ADAPTED TO LONG-TERM PRISM REVERSAL OF VISION. G. Melvill Jones and P. Davies*. Aviation Medical Research Unit, Dept. of Physiology, McGill Univ., Montreal, Canada H3G 1Y6.

Two cats, acting in series as test and control animals, have been adapted to 300 days of continuous horizontal reversal of vision using polystyrene dove prisms held in a skull-mounted mask of ultra light-weight plastic construction. VOR adaptation was measured in terms of (i) phase (relative to normal) and (ii) gain (eye angle/head angle) of the reflex response to sinusoidal rotation in the dark. Using a new computerised method of analysis both these parameters were found to be profoundly affected by adaptation to vision reversal. With optimal stimulus conditions both animals demonstrated effective VOR reversal. However the adapted response proved highly dependent upon both amplitude and frequency of the sinusoidal stimulus.

Quantitative analysis of these non-linearities in the first animal has thus far revealed the following results: (i) At 1/8 Hz, increasing the velocity amplitude from 8 to 91°/sec peak to peak led to eventual restoration of phase to normal, but with much reduced gain. (ii) At 17°/sec peak to peak amplitude, the reduction of frequency from 0.25 to 0.03 Hz led to change of phase from near reversal to near normal, with gain always less than normal. A simple neural model previously proposed to account for more limited results from human subjects is readily updated to account for these non-linearities.

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1190 FUNCTIONAL ORGANIZATION OF CAT LATERAL GENICULATE NEURONS FOLLOWING NEONATAL ABLATION OF VISUAL CORTEX. R.E. Kalil and E.H. Murphy. Dept. of Anat., Univ. Wis., Madison, WI. and Biopsychology Section, Univ. Chicago, Chicago, IL. 60637.

In the adult cat, most of the cells in the dorsal lateral geniculate nucleus (LGN) undergo retrograde degeneration following removal of visual cortex. In contrast, if visual cortex is ablated in neonatal kittens, there is a marked sparing of neurons throughout the LGN. Fink-Heimer and autoradiographic studies suggest that LGN cells spared in these cats continue to receive input from the retina. To investigate the functional organization of surviving geniculate cells we have recorded from single neurons in the LGN of adult cats with ablations of visual cortex made on the day of birth. In general, these cells had receptive fields with a concentric center-surround organization characteristic of normal LGN neurons. However, centers and surrounds were consistently larger than normal, often by several orders of magnitude. Furthermore, some cells could be influenced by a spot of light presented at almost any location within the contralateral hemifield. Grossly, the retino-geniculate map appeared topologically correct, but it was not uncommon to record in a single penetration from cells with receptive fields widely separated from each other. Thus in infant operated animals functional retino-geniculate synapses can be demonstrated, although it appears that a reorganization has occurred which involves extensive convergence, and possibly scrambling of retinal afferents.

1191 ATTENUATION OF RECOVERY OF MOTOR FUNCTION AND BRAIN DOPAMINE IMBALANCE AFTER UNILATERAL LH AND SN LESIONS IN THE RAT USING A NOVEL BEHAVIORAL TASK. M. Kanner, Dept. of Psychiatry, Univ. of Chicago Pritzker School of Medicine, Chicago, Ill. 60637

Sensorimotor dysfunction in bilateral and unilateral lateral hypothalamic (ULH) rats has been extensively studied by Marshall et al (1971). Here, to determine the role of bioamines in the mediation of the recovery of motor function, ULH or sham lesions (SLH) were made and, as expected, produced only a transient effect on feeding but severe motor dysfunctions. Food intake was evaluated by the postoperative placement of food pellets in wire baskets hung from the outside of the home cage vs pellets placed on the cage floor. All rats had prior experience with basket feeding. Motor ability tests were performed. ULH-wall rats were significantly ($p<.05$) retarded in several forms of motor recovery (step-down onto 4 paws in home cage, general locomotion, grooming, deviating to lesioned side, etc.) by 1-3 days over ULH-floor or SLH-wall rats. They were also hyperreactive to sensory stimuli, slower to regain eating and drinking and to stabilize body weight. To assess the role of the striatonigral dopamine (DA) system unilateral substantia nigra (USN) rats or shams (SSN) were studied and found to have similar deficits. USN-wall rats showed motor recovery retardation over USN-floor and SSN-wall rats. It is known that DA is decreased more than NE on the lesioned side of these rats. While depletion of total brain CA's appears to be an important variable in the plasticity of the brain after lesion regardless of whether it is bilateral or unilateral, the data presented here indicate that unilateral DA depletion on the lesioned side of the brain is of sufficient magnitude to attenuate the recovery of motor function. A DA imbalance in the brain may be important in the reorganization of the brain after damage and especially in the recovery of motor function. (Supported in part while M.K. was a Fellow of the Foundations' Fund for Research in Psychiatry).

1192 THE CONTROL OF NERVE TERMINAL SPROUTING IN THE CARDIAC GANGLION OF THE FROG. Chien-Ping Ko* and Stephen Roper. Dept. Anat., Univ. Colo. Med. Center, Denver, Co. 80220.

When half the preganglionic nerve (vagus) supply to the parasympathetic cardiac ganglion in the frog is destroyed, the remaining intact vagal fibers proliferate, or "sprout" and within 8-10 days establish functional connections with the entire population of neurons. What factors are important in initiating this striking reorganization of synapses is not as yet known. We are testing whether cessation of propagated impulses or axoplasmic transport in one nerve triggers synaptic terminal sprouting in other nerves. Drugs were applied chronically to intact vagus nerves by mixing the agent with liquid Silastic A RTV (Dow Corning) silicone rubber allowing the amalgam to set into a flexible cuff, and implanting it around the nerve. Cuffs containing 1-5% (by weight) tetrodotoxin (TTX) effectively blocked impulse propagation for up to one week when implanted around the nerve. Ganglia from experimental animals were dissected free and neurons impaled with microelectrodes to record synaptic responses evoked by stimulating the left, the right, or both vagus nerves. There was no sprouting or other change in the organization of synapses in the cardiac ganglion when TTX-impregnated cuffs were implanted around one nerve for up to 10 days. Colchicine and vinblastine-impregnated cuffs were applied to nerves to examine the contribution of axoplasmic transport to sprouting. When vinblastine cuffs (1-2% by weight) were implanted around the right vagus nerve and the left nerve crushed, sprouting did not take place in the vinblastine-treated right vagal fibers. As one might expect, axoplasmic transport is necessary for the proliferation of synapses to occur. We are now examining whether blocking axoplasmic transport in one nerve initiates sprouting in the other. Supported by NIH, Mallinckrodt Found., and Colo. Heart Assoc. grants.

1193 SELECTIVITY OF EXPANDED RETINAL PROJECTIONS FOR SUBCORTICAL VISUAL NUCLEI. P.W. Land, E.H. Polley and M.M. Kernis. Depts. of Anat., Ophthal. and Neurosurg., Univ. of Ill. at the Medical Center, Chicago, Illinois 60612.

As part of an ongoing study of modified retinal projections in rats with unilateral congenital eye defects, we previously described expanded projections to the dorsal lateral geniculate nucleus and superior colliculus. We have now extended our investigation to include a description of the patterns of retinal projections to other subcortical visual centers. The intact eye of adult rats with unilateral anophthalmia or microphthalmia (Land, et al., 1976) was removed under Nembutal anesthesia. Following a 5 day survival, animals were perfused with 4% paraformaldehyde/1% glutaraldehyde in Sorensen's buffer (pH 7.3). Serial transverse sections through the brains were stained by Method I of Fink and Heimer (1967) or with cresyl violet. Contralateral retinal projections to the ventral lateral geniculate nucleus (LGNv), pretectal nuclei and nuclei of the accessory optic tract were in general agreement with previous reports. We have, however, failed to confirm a projection to the lateral posterior nucleus in these animals or in normal controls. Expanded fields of terminal degeneration were observed in the ipsilateral LGNv, olivary pretectal and posterior pretectal nuclei. These nuclei normally receive a limited ipsilateral projection. In addition, degenerating terminals were evident in the ipsilateral nucleus of the optic tract (NOT) and in the dorsal, lateral and medial accessory optic nuclei. These results indicate: 1) a remarkable degree of selectivity of developing retinal projections for subcortical visual nuclei even when these projections are "expanded" or occur in the inappropriate side of the brain; 2) not all expanded ipsilateral retinal projections in animals with unilateral eye defects can be attributed to post-chiasmatic branching of the normal complement of retinal axons, since the accessory optic nuclei and NOT do not receive ipsilateral projections in the normal animal.

1194 EFFECTS OF EARLY HIPPOCAMPAL DAMAGE IN MONKEYS, Helen Mahut and Stuart Zola, Psychology Dept., Northeastern Univer., Boston, Mass., 02115.

We investigated the effects of early fornix sections on 3 tasks sensitive to equivalent damage in older monkeys. The latter are impaired on spatial reversals, facilitated on object reversals and impaired on successive retentions of visual discriminations. Infants were operated between 42 and 82 days of age. Five unoperated infants and corresponding groups of juveniles served as controls. Post-operatively, infants were impaired on the spatial but not on the object reversal task. At 1 yr of age, they were still impaired on the spatial task. Though as a group, they had normal scores on the object task, delayed lesion effects were revealed which were significantly correlated with age at surgery: deleterious effects (facilitation of performance) appeared only if it was performed at 60 days, or later. Retention was impaired both post-operatively and at 1 yr. The results have direct bearing on the problem of plasticity in limbic structures and the non-unitary nature of hippocampal function.

1195 EFFECTS OF COMBINED NEONATAL ENUCLEATION AND VISUAL CORTEX ABLATION ON THE SUPERIOR COLLICULUS OF THE RABBIT. Lawrence H. Mathers, Jr. Dept. Anat., Sch. Med., Stanford Univ., Stanford, CA. 94305.

Dutch-belted rabbit pups were subjected to monocular enucleation and removal of large regions of the contralateral visual cortex on the day of their birth. After survival of 3-9 months, they were anesthetized and sacrificed. Tissues were taken from the central region of the superior colliculus and processed for electron microscopic examination. The overall thickness of the superficial gray laminae of the superior colliculus was reduced from 700-800 micra to 350-450 micra. The stratum opticum was reduced from its normal 200-300 micra to 150-200 micra in thickness. Cells throughout the superficial gray lamina were reduced in cross-sectional area by 20%, and the number of myelinated axons in the upper gray laminae was markedly reduced. Synaptic arrangements were grossly distorted. While a large number of asymmetric synapses involving small presynaptic axons was persistent, approximately 15% of the asymmetric postsynaptic densities seen was attached to an abnormal presynaptic profile. These included dendrites, somata, glia and myelin sheaths. A larger percentage of serial symmetric synapses involving multiple F profiles was also present. These results indicate that the extent of abnormal synaptogenesis in developing systems is dependent not only upon the age at which lesions are made, but also on the total number of presynaptic profiles removed. (Supported by NIH NS 11669).

1196 INVOLVEMENT OF COATED VESICLES IN SYNAPTIC REINNERVATION OF THE PARTIALLY DEAFFERENTED DENTATE GYRUS OF THE ADULT RAT. J.R. McWilliams* and G.S. Lynch. (SPON: J. Wells). Dept. Psychobiology, U.C. Irvine, Irvine, C.A. 92715.

Coated vesicles have been implicated in synaptogenesis in the developing cerebellar cortex (Altman, Br. Res. 30:311, 1971) and in tissue culture explants (Rees, Bunge and Bunge, J. Cell Biol. 68:240, 1976). We have suggested the involvement of coated vesicles in reinnervation in the Dentate Gyrus following degeneration induced by a commissural lesion (McWilliams, Lynch, and Cotman, Neurosciences, 1975). Since then we have investigated the time course of the reinnervation and have quantified the number of coated vesicles and vacant postsynaptic densities (psd's) in the region of deafferentation.

Reinnervation begins approximately two weeks postlesion and continues until a near normal number of synaptic boutons is restored by 6 weeks. The number of coated vesicles begins to increase 3-4 days following the lesion; a 20-50% increase occurs prior to the onset of reinnervation. Along with this increase, the number of vacant psd's increases two to threefold. These quantifications and observations suggest that: 1. upon denervation, the number of coated vesicles increases in the region of partial deafferentation, and these vesicles appear to be synthesized in the Golgi apparatus and the smooth endoplasmic reticulum of the cell body and dendrite; 2. some psd's which lose their presynaptic elements detach from the dendritic membrane and become coated vesicles; 3. the attachment of the coated vesicles to the dendritic membrane appears to initiate the formation of the psd's, and in most cases, this appears to precede the formation of the presynaptic element.

1197 HISTOCHEMICAL EVIDENCE OF POST-LESION AFFERENT REMODELING IN THE HIPPOCAMPAL FORMATION OF THE MATURE CAT. J. A. Messenheimer * and O. Steward. Depts. Neurol., Neurosurg., and Physiol. (SPON: N. H. Bass), Univ. of Va. Sch. Med., Charlottesville, Va. 22901

Axonal sprouting has been demonstrated in a variety of brain regions following lesions in adult rats. However, the occurence of similar postlesion remodeling in mature animals of species other than rat remains an open question. Mature rat hippocampus responds to ipsilateral entorhinal lesions with an intensification of acetylcholinesterase (AChE) staining in the deafferented portion of the dentate gyrus (Lynch,Matthews, Mosko, Parks,Cotman, Brain Res. 42: 311, 1972). Since AChE in this region is normally localized within fibers arising from the medial septal nucleus, it has been suggested that such intensification of staining represents sprouting of septal afferents. Similar increases in choline acetyltransferase support the interpretation that this intensification of AChE activity reflects sprouting of cholinergic septal afferents in the deafferented region (Storm-Mathisen, Brain Res. 80: 181, 1974). We have investigated the possibility that similar changes might occur following unilateral entorhinal lesions in mature cats. Cats received unilateral entorhinal lesions through temporal craniotomies and were sacrificed at varying post-operative intervals. Brains were removed, frozen, and sectioned at 40-80μm prior to AChE histochemical staining with a modification of the method of Koelle. Intensification of AChE staining was observed in the dendritic region normally occupied by entorhinal afferents in a pattern equivalent to that observed in the adult rat subjected to entorhinal lesions. If these changes in AChE activity are indeed indicative of postlesion axonal sprouting, the hippocampus of the mature cat is equally capable of this plasticity as the mature rat. (Supported by USPHS RESEARCH GRANT NO. 1 R01 NS12333 to O. Steward).

1198 EFFECT OF PRIOR LESION ON RECOVERY FROM LATERAL HYPOTHALAMIC DAMAGE. L. J. Misantone. Dept. Anat., Hahnemann Med. Coll. Phila., Pa., 19102.

Unilateral damage to the lateral hypothalamus (LH) causes weight loss which is probably due to interruption of the nigrostriatal dopamine (DA) projection along its course (Ungerstedt,1971). Neuroplastic phenomena such as denervation supersensitivity or collateral sprouting induced by frontal cortex (FC) lesions might underlie protection from weight loss after LH lesions (Glick and Greenstein, 1972). Because both nigral and FC projections terminate in the neostriatum, this could be an area in which these neuroplastic responses occur. Since serotonergic cells of the dorsal raphe nucleus (DR) also project to the neostriatum, prior destruction of DR might ameliorate the effects of LH damage on ingestive behavior. Adult Sprague-Dawley female rats were subjected to either sham operation (SH) or electrolytic damage to DR. Unilateral LH lesions were made in 8 DR and 7 SH rats 28 days (ds) following surgery. DR and SH groups were equivalent in total food and water intake on ds 25-28, and also in body weight on d 28. They showed similar body weight losses from the 28 d level to that measured 3 ds after LH lesions (DR = -30.5 $\pm$ 17.4 g; SH = -30.6 $\pm$ 22.9 g). In addition, equivalent depressions in both groups of total food and water intakes for ds 1-3 after LH lesion, compared to ds 25-28, were observed. Forebrain tissue rostral to the retrochiasmatic region of 5 DR and 5 SH rats without LH lesions was assayed for DA content at 28 ds. Forebrain DA level of the DR group was not elevated over that of the SH group (DR = 2.369 $\pm$ .421 ug/g; SH = 2.447 $\pm$ .197 ug/g), suggesting that sprouting of DA fibers did not occur. The lack of protective effect from LH lesions by prior DR damage suggests that supersensitivity was not induced. These results point out that protection from LH weight loss by prior lesion may be system specific. (Supported by a Grant from Sigma Xi)

1199 IRRITATION AND DEPRESSION OF SINGLE NEURONS NEAR AN ELECTROLYTIC LESION. James C. Pittman*, Dennis M. Feeney and Maura D. Spiker*. Depts. Psychol. & Physiol., Univ. New Mexico, Albuquerque, N.M. 87131 U.S.A.

Spontaneous action potentials were recorded by tungsten microelectrode from a 4x4x5 mm region of anterior thalamus in awake chronic cats before and after making an anodal electrolytic lesion at the posterior edge of the region. The lesion involved nucleus centre median. Electrode tracks were spaced systematically at distances 0.5 to 4.5 mm from the lesion site. Ten tracks were made in 3 sessions before the lesion. Thirty tracks were made in 8 sessions spaced from 1 day to 31 days after the lesion.

After the lesion there was a dramatic reduction in the number of cells encountered per track in a wide area outside the histologically defined lesion. Apparently cells are depressed over a wide area near the lesion and tend to recover over time. Thus the effective size of a lesion may be considerably larger than is apparent from histological examination.

After the lesion, cells were encountered which had distinctly abnormal firing patterns: they had interspike intervals as short as 1 msec and fired in brief stereotyped bursts and/or tonic high frequency discharge for several seconds. Abnormal cells tended to be found in clusters on the fringe of the lesion with normal cells above and below along the length of the track. Abnormal cells were found as early as 2 and as late as 31 days post-lesion. These abnormal firing patterns may be the result of irritation.

Transient depression and irritation of cells are processes which act on neurons outside the histologically defined lesion area and may interact in contributing to the effects of and recovery from electrolytic lesions.

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1200 RUBROSPINAL TRACT SPROUTING ROSTRAL TO SPINAL LESIONS IN THE RAT. J. Prendergast and L.J. Misantone, Depts. Anat., Med. Coll. of Pa. and Hahnemann Med. Coll., Phila., Pa. (Supported by NIH #NS11919)

Axonal sprouting by descending systems into several regions of the gray matter rostral to mid-thoracic spinal hemisection has been reported in adult rats whose cords had been lesioned either as newborns (0-3 d.old) or as weanlings (21-25 d.old)(Prendergast and Stelzner,'76). The sprouting may be a response to denervation of the gray matter, rostral to the thoracic lesion, due to retrograde axonal degeneration or to loss of ascending propriospinal fibers or both. In an attempt to identify possible systems contributing to the sprouting, the rubrospinal tract (RST) was investigated. Unilateral lesions of various sizes were placed in the thoracic cords of newborn and weanling rats. By 90 d. the degeneration in the gray matter resulting from the thoracic lesion had disappeared. 5 to 6 months after the thoracic lesion, the decussation of the RST was destroyed electrolytically, and the cords prepared with the Fink-Heimer technique. Degeneration of the RST on the experimental (lesioned) side was confined to a smaller, more compact bundle containing fewer fibers than on the control side. Degeneration in the terminal fields of the RST 1-1½ cm rostral to the thoracic lesion nevertheless showed an increase in density of degenerating debris on the experimental side of the cord. In both the newborn and weanling operates the degeneration was found mainly in Rexed's laminae V and VII, regions normally innervated by the RST. Thus in developing animals with spinal lesions the remaining RST axons rostral to the thoracic lesion sprout an increased number of collaterals into the gray matter. These collaterals appear to be locus-specific in that they do not invade other denervated regions of the spinal gray matter (e.g. ventral horn). This is true whether the thoracic lesion be confined to the dorsal lateral funiculus or if it involved most of the white matter.

1201 RECOVERY OF FUNCTION AFTER HIPPOCAMPAL VASCULAR DAMAGE IN MICE. Steven A. Reid*, C.A. Boast*, and S.F. Zornetzer (SPON: K.M. Heilman). Dept. Neurosci., Coll. Med., U. Florida, Gainesville, FL 32610.

We have previously reported that ferric ions (Fe^{+3}) associated with electrodes implanted in the mouse hippocampus are correlated with a deficit in 1-trial inhibitory avoidance behavior. When iron-free electrodes were implanted into the hippocampus a behavioral deficit was observed and correlated with Fe^{+3} located bilaterally in the dentate gyrus. Electron beam microprobe analysis verified that the previously observed histochemical reaction product accurately reflected the presence of iron closely associated with electrode tracks. Thus, the Fe^{+3} must be of biological origin and were hypothesized to be the result of electrode-produced vascular damage. India ink perfusion techniques were used to study the microvasculature of the hippocampus in normal and operated mice. Fe^{+3} were observed in the tissue and were associated with damaged blood vessels lying in the hippocampal fissure. Thus, the observed Fe^{+3} were of vascular origin and the associated behavioral deficit may be the result of these localized vascular disruptions.

In the present experiment an extended recovery period (46-85 days) following the production of discrete hippocampal vascular damage in Swiss/ICR mice resulted in no impairment in inhibitory avoidance behavior. Thus, recovery of function can occur following localized cerebrovascular damage in the hippocampal formation.

Our findings suggest that a better understanding of local cerebrovascular networks will lead to a better understanding of localization of function. Further, localized vascular insult in the mouse dorsal hippocampus should prove useful as an animal model for understanding the basis of human cerebrovascular disorders.

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1202 DISPLACEMENT OF SPROUTED SYNAPSES BY REGENERATING AXONS IN FROG CARDIAC GANGLION. Stephen Roper and Chien-Ping Kó* (SPON: R. Lasher). Dept. Anat., Univ. Colo. Med. Center, Denver, Co. 80220.

It has long been recognized that synaptic connections which remain intact after damage to nervous tissue "sprout" into the denervated areas. What controls are exerted on the sprouting and how permanent are sprouted synapses is not yet known. We have been studying these problems in the cardiac ganglion of the frog. When the left vagus nerve (preganglionic supply) is crushed, vagal fibers in the right nerve sprout and innervate 100% of the ganglion cells. Normally about only half the cells receive input from the right vagus nerve. This is demonstrated by recording intracellular responses to vagal stimulation in ganglion cells from normal and operated animals (Courtney & Roper, Nature 259:317,1976). We have examined regeneration of vagal fibers and the fate of sprouted synaptic connections at long intervals after crushing the left vagus nerve. If ganglion cells are impaled 5-6 weeks after the operation, stimulation of regenerating axons evokes synaptic responses in cells which are innervated by the sprouted right vagus nerve. At later stages of regeneration (8-60 weeks) there is an increase in the number of cells which receive left (regenerating) inputs and a concomitant decline in the innervation by the sprouted right vagus nerve. When synaptic boutons are stained with zinc iodide and counted, the number of synapses per cell body remains constant during regeneration of left vagal fibers (control = 9.1 boutons/cell, regenerated = 8.8). These findings suggest that right vagal endings are being displaced by regeneration of the original innervation. Because transmission at sprouted and at normal synapses was indistinguishable, we cannot determine yet whether sprouted vagal endings are preferentially being displaced during regeneration. Supp. by NIH, Mallinckrodt Found. and Colo. Heart Assoc.

1203 ALTERED PATTERNS OF INNERVATION IN FROG MUSCLE AFTER DENERVATION. Shlomo Rotshenker* and U.J. McMahan* (SPON: S.W. Kuffler). Dept. Neurobiol., Harvard Medical School, Boston, MA 02115

The pattern of reinnervation of muscle fibers after a nerve crush was examined in the cutaneous pectoris muscle of the frog by microscopy and electrophysiology. Normally, about 16% of the muscle fibers are innervated by more than one motor neuron. Two months after reinnervation, 50% of the fibers are polyneuronally innervated and this high incidence persists for at least seven months. Although the number of inputs to individual muscle fibers is increased after regeneration, the number of motor neurons innervating the muscle as well as the number of muscle fibers comprising the muscle is the same as normal. Regenerated axons contact muscle fibers precisely at the original synaptic sites and the terminal branches from different axons that end on the same muscle fiber often run side by side occupying stretches of original postsynaptic membrane normally covered by one terminal. These findings indicate that the number of inputs to reinnervated frog muscle fibers is limited by the amount of vacant original postsynaptic membrane and the ability of the regenerating axons to reach it. An additional observation was that control cutaneous pectoris muscles situated contralateral to those that were denervated exhibited a somewhat higher incidence of polyneuronal innervation (27%) than muscles in normal animals. The most obvious explanation is that denervation of one cutaneous pectoris muscle can alter the pattern of innervation in the other.

1204 ADULT ONSET MONOCULAR PARALYSIS PRODUCES SELECTIVE LOSS OF LATERAL GENICULATE CELLS WHICH RECEIVE SLOWLY CONDUCTING RETINAL AFFERENTS. W. L. Salinger, M. A. Schwartz, and P. R. Wilkerson*. Dept. of Psych., UNC-Greensboro, N.C. 27412.

Receptive field analyses of X- and Y-cell frequency in the lateral geniculate nucleus (LGN) indicate that chronic monocular paralysis (>13 days) produces selective loss of X-cells in layers receiving from the paralyzed eye. The effects of monocular paralysis (MP) on geniculate layers which receive from the mobile eye were not assessed in earlier experiments. The experiment reported here re-examined the effects of chronic MP by measuring the response latencies to optic chiasm (OX) shock in LGN cells. This technique permits comparison of the effects of chronic MP on LGN cells receiving input from the mobile eye with those receiving input from the paralyzed eye. Conduction latencies from OX to single cells in the LGN were recorded from four acutely (<5 days) paralyzed cats and compared to those recorded from four chronically (>13 days) prepared animals. Contralateral LGNs of cats with chronically paralyzed left eyes had diminished numbers of cells with long latency responses to OX shock, thus supporting previous reports of a loss of X-cells following chronic MP in adult cats. Here the obtained loss was more pronounced among cells driven by the mobile eye (lamina A_1) than among cells receiving inputs from the paralyzed eye (laminae A & B). The unexpected observation that lamina A_1 cells (mobile eye) are more strongly effected by the chronic paralysis than laminae A and B cells (paralyzed eye) strongly implicates post-chiasmic mechanisms in the genesis of these changes.

1205 FORMATION OF SYNAPSES BETWEEN PARASYMPATHETIC NEURONS FOLLOWING LOSS OF PREGANGLIONIC SYNAPTIC INPUT. Péter B. Sargent* and M. J. Dennis* (SPON: Roy H. Steinberg) Depts. Physiol. Biochem., School of Medicine, University of California, San Francisco, CA 94143

Parasympathetic ganglion cells in the interatrial septum of the frog heart are innervated by axons from the vagus nerves. Action potentials can be elicited from these ganglion cells orthodromically, by vagal nerve stimulation, and antidromically, by stimulation of the nerve trunk through which their axons project to ventricular muscle. Upon resection of the vagus nerves preganglionic input is eliminated for up to three months, and one can test for "intrinsic" synaptic connections between ganglion cells by recording intracellularly during antidromic stimulation. Two to five days after vagus resection 2% of the ganglion cells (3 of 123 cells, from 5 septa) received intrinsic synaptic connections. This suggests that the incidence of these synapses is very low in normally innervated septa. However, after denervation for 50-80 days, 30% of the ganglion cells (30 of 99 cells, from 10 septa) received intrinsic excitatory input. The inference that this synaptic input is derived from other ganglion cells is supported by examples in which direct electrical stimulation of the soma of one neuron elicited a synaptic potential in another. Prolonged loss of normal preganglionic vagal input thus leads to the formation of synapses between ganglion cells. (Supported by USPHS Grants Nos. NS 00303 and NS 10792.)

1206 CUMULATIVE EFFECTS OF SEQUENTIAL LESIONS ON AXON SPROUTING IN THE HIPPOCAMPUS. Stephen Scheff*, Larry Benardo*, Carl Cotman, and Gary Lynch. Dept. Psychobiol., Sch. Biol. Sci., UCI, Irvine, CA. 92717.

The present study investigates whether or not sequential lesions of the entorhinal cortex can increase the rate of axon sprouting compared with a single stage lesion. Entorhinal lesions performed in a single stage result in an outgrowth of the commissural-associational system into the overlying entorhinal zone. Previously it has been observed that the commissural-associational fiber plexus spreads approximately 30% in response to the single stage entorhinal lesion and the spread requires 6-7 days.

Adult albino rats were subjected to sequential lesions of the entorhinal cortex and allowed to survive for various inter-lesion-intervals. Animals received a small priming lesion, which in itself caused little or no fiber growth into the entorhinal zone, followed by a complete entorhinal lesion. Sections were stained with the Holmes fiber stain to evaluate fiber growth. In animals with priming lesions of the medial entorhinal cortex, the commissural-associational fiber plexus requires only about one half the time required with only a single stage lesion. These results suggest that the reactions responsible for controlling the spreading of fibers can prime the sprouting reaction. It is well known that animals with serial lesions in behavioral studies recover more rapidly. Our results may explain, in part, behavioral data indicating that animals with serial lesions recover more rapidly than animals with single stage lesions. (Supported by NIMH Research Grant, MH 19691)

1207 INFLUENCE OF PATTERNED VISUAL EXPOSURE ON LATERAL GENICULATE CELL LOSSES PRODUCED BY CHRONIC MONOCULAR PARALYSIS. M. A. Schwartz, W. L. Salinger, and P. R. Wilkerson*. Dept. of Psych., UNC-Greensboro, N.C. 27412.

Receptive field analyses of the effect of chronic monocular paralysis (>13 days duration) indicate a selective loss of X-cells in the lateral geniculate nucleus (LGN). Chronic monocular paralysis (MP) also produces a significant loss of cells with long latency responses to OX shock. While observed in layers receiving from both the paralyzed (A&B) and mobile (A_1) eye, this latter effect is most pronounced in the A_1 layer receiving from the mobile eye. The selective loss of long latency cells could result either from the effects of MP *per se* or from the resulting discordant binocular input. The experiment reported here measured the effects of MP on the LGN in chronically prepared cats not permitted pattern vision after paralysis. These results were compared with data obtained from cats permitted pattern vision after paralysis. Comparison of the two indicates that MP produces a more extensive loss of slowly conducting cells in animals exposed to patterned visual stimuli following paralysis. MP in the absence of exposure to patterned stimulation does, however, produce a smaller, though significant, loss of LGN cells with slowly conducting retinal afferents. Taken together, these results suggest that the loss of slowly conducting cells produced by chronic MP arises in part from the discordant visual input created by oculomotor imbalance, and in part from the oculomotor imbalance itself.

1208 EFFECT OF CONTINUOUS LIGHT EXPOSURE ON RETINOTECTAL COMPRESSION AFTER EXCISION OF CAUDAL TECTUM IN GOLDFISH. Margaret Y. Scott* and Ronald L. Meyer* (SPON: A. Van Harreveld). Division of Biology, California Institute of Technology, Pasadena, CA. 91125.

Following excision of the caudal half of the optic tectum in goldfish, the caudal optic fibers deprived of normal target zones, come to project after several months, onto the residual rostral half tectum. An orderly but compressed topographic projection of whole retina on rostral half tectum results even when the optic nerve is left intact. Retinotectal compression has been reported to be inhibited, as judged by postsynaptic electrophysiological recording, by keeping fish in continuous light following surgery (Yoon, J. Physiol. 246: 673, 1975). This finding was further tested in the present experiments in goldfish by removing the caudal half tectum and maintaining them postoperatively in continuous 24 hr lighting. Tests for recovery of vision employed both behavioral and electrophysiological mapping procedures and were made at various postsurgical intervals. Inhibition of compression was not found under our conditions for reasons not yet clear. Behavioral mapping of the visual field using a conditioned respiratory response to visual stimuli presented along the horizontal meridian showed orderly restoration of vision in the formerly blind field. Vision gradually expanded from the central edge of the scotoma into the blind posterior field until complete recovery was achieved in about 3 months, in approximately the same time course described by the first author for regular diurnal light (Scott, Anat. Rec. 181: 474, 1975). An eye-in-water physiological method for mapping terminal arborizations of optic axons also showed a similar sequential rostrocaudal recovery - restoration being essentially the same time course, and showing an orderly compressed retinotectal projection. (MYS supported by USPHS Grant GM00086 and Evelyn Sharp Graduate Fellowship; RLM by Hixon Fund and NIMH Grant MH03372 to R. W. Sperry.)

1209 RADIOAUTOGRAPHIC, ELECTROPHYSIOLOGICAL AND BEHAVIORAL ANALYSES OF OPTIC NERVE REGENERATION IN GOLDFISH. A.D. Springer, A.M. Heacock and B.W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

Rate of recovery of vision in goldfish following optic nerve crush and unilateral enucleation was found to be temperature dependent in fish maintained at 30°C, 25°C, or 20°C. Regeneration at 30° appeared to be complete 12 days postoperatively on the basis of radioautography of the tectum. However, the first indication of restored vision, which consisted of a respiratory deceleration to a change in illumination, occurred 15 days (median) postoperatively. Ability to localize food was seen at 17 days, while the optomotor response appeared at 28 days. Biochemical analysis and radioautographs of the tectum revealed that the remaining optic nerve had regenerated to both tecta. Fibers reached the ipsilateral tectum (IOT) via the ipsilateral tract at the chiasma. Comparable debris in both tracts was not sufficient in producing an IOT projection since each nerve innervated a contralateral optic tectum (COT) when both nerves had been crushed simultaneously. When the arrival of one nerve at the chiasma was delayed by staggering the nerve crushes, the nerve that first arrived at the chiasma partially innervated the IOT. Electrophysiological analysis indicated that in most instances a small percentage of fibers from each area of the retina innervated the IOT topographically. In order to determine whether the IOT projection was behaviorally functional, the COT was ablated. All fish failed to respond to changes in illumination as measured by respiration and also failed to swim with or against the stripes in an optomotor drum. Thus the IOT input, possibly because of its sparseness, could not be shown to be functional by either test. (Supported by grants NSF-BMS-75-0381 and MH12506-10).

1210 POST-OPERATIVE CELL PROLIFERATION IN THE GOLDFISH OPTIC TECTUM.
James A. Stevenson* (SPON: I.A. Meinertzhagen). Dept. of Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada

While much attention has been paid to the degenerative and regenerative activities of retinal ganglion cell axons after optic nerve (ON) section in goldfish, little has been paid to the concurrent activities of cells in the optic tectum. This study investigates cell proliferation in the goldfish tectum after ON section.

To study the time course of proliferation, one ON was crushed and the other left intact in 20 goldfish. At 5 day intervals following surgery, pairs of fish were injected with ^{3}H-thymidine and sacrificed 2 days later. Those fish labelled 30-35 days after ON crush had significantly more labelled cells, of a particular type, in the tectum contralateral to the crushed nerve than in the control tectum. These newly formed cells appeared to be neurons and were situated centrally to the densely packed cells of the stratum periventriculare.

To investigate the conditions under which this proliferation takes place, 30 fish had one ON crushed and the contralateral eye enucleated. At 30, 35 or 40 days following surgery, these fish were injected with ^{3}H-thymidine; post-injection survival times ranged from 2 hours to 12 weeks. If the proliferation were caused by denervation of the tectum, the number of labelled cells should be the same in both tecta. If the proliferation occurred in response to the reinnervation of the tectum, there should be more labelled cells in the reinnervated tectum. The latter is true; the number of labelled cells was greater in the reinnervated tectum.

These results suggest that some cells in the goldfish tectum possess proliferative capabilities typically seen in embryonic nervous systems. The expression of these capabilities is greatly enhanced when the tectum is reinnervated by ON fibers.

1211 CELLS OF ORIGIN OF CONTRALATERAL ENTORHINAL EFFERENTS WHICH REINNERVATE THE DENTATE GYRUS OF THE RAT FOLLOWING IPSILATERAL ENTORHINAL LESIONS.
Oswald Steward. Depts. Neurosurg. and Physiol., Univ. of Va. Sch. Med., Charlottesville, Va. 22901

Granule cells of the rat dentate gyrus which are denervated by unilateral entorhinal cortical (EC) lesions are reinnervated in part by proliferation of surviving contralateral entorhinal pathways. If this lesion-induced circuit originated from the same cell type in the EC as did the normal connections, then the post-lesion growth might restore a specific type of input to the denervated dentate gyrus. To investigate this question, horseradish peroxidase (HRP) was used to identify the cells of origin of the normal ipsilateral and the lesion-induced crossed pathways. A 50% solution of HRP was injected into the normal and the reinnervated dentate gyrus. In normal animals the almost exclusively ipsilateral projections from the EC to the dentate gyrus originate from stellate cells in layer II while the bilateral projections to regio superior of the hippocampus proper originate from medium sized pyramidal cells of layer III (O.Steward & S.Scoville,in press). Thus, ipsilateral to an HRP injection in normal animals, labeled cells were found in layers II & III, while contralaterally, the label was localized almost exclusively to cells in layer III. The few cells which were found in layer II were very lightly labeled. Injections into the reinnervated dentate gyrus, however, resulted in a very conspicuous, heavily labeled population of cells in layer II of the EC contralateral to an injection. Since labeled cells in layer II contralateral to an injection are rare, and only very lightly labeled in normal animals, but are conspicuous in animals with long standing unilateral EC lesions, this suggests that these cells give rise to at least some of the fibers which reinnervate the granule cells. These are the same type of cells (stellate cells) which normally project to the ipsilateral dentate gyrus. (Supported by USPHS Research Grant No. 1 R01 NS12333).

1212 CANDIDATE MECHANISMS OF PLASTICITY IN THE HIPPOCAMPUS. T.J. Teyler and B.E. Alger*. Dept. Psychol., Harvard Univ., Cambridge, Ma. 02138.

The hippocampal and dentate gyri have been shown to display several forms of plastic alteration of synaptic efficacy ranging from short-term to long-term phenomena. The Ca1 region of the hippocampal explant was studied electrophysiologically in an attempt to characterize the candidate mechanisms underlying these plastic alterations. Two seperate fiber systems, afferent to the CA1 sub-field, were identified: one in stratum oriens, the other in stratum radiatum. Both inputs have excitatory connections to the CA1 pyramids and display long-term potentiation.

Double-shock stimulation to either path showed an inhibition of the second response at short intervals (10-40msec) and facilitation at longer intervals (40-150msec). Double-shock stimulation across paths, when not contaminated with antidromic activation of CA1 cells, showed the inhibition at short intervals but failed to show crossed facilitation. Test pulses to the same path or across paths, following (1-20sec) the application of a brief tetanic stimulus, showed post-tetanic-potentiation in both cases. Finally, long-term-potentiation was established in the two paths in alternating order (low-frequency stimulation first to one path, then the other, etc.). No interaction was observed between the paths. Thus, the short-term phenomena of crossed post-tetanic-potentiation is observed, whereas crossed facilitation is not seen. No crossed interaction is observed during long-term potentiation.

Arguments are presented that: a) presynaptic factors are not mediating these effects, and that neither b) the activity of hypothetically present interneurons nor c) a generalized change in post-synaptic excitability can account for all of the observed effects. Alternative mechanisms are discussed in the light of the results from intracellular recordings of CA1 neurons during induced plastic changes.

1213 PROGRESSIVE ALTERATIONS IN OPTIC TRACT AND RETINOTECTAL TOPOGRAPHY DURING OPTIC NERVE REGENERATION IN RANA PIPIENS. Susan B. Udin. Psychology Dept. and Res. Lab. of Electronics, MIT, Cambridge, MA 02139.

The reestablishment of normal retinotectal topography after optic nerve transection in frogs was studied using microelectrode recordings from optic nerve arborizations in the tectum.

Normally, optic nerve fibers enter the dorsal tectum along its rostral margin and travel caudally. Activity of terminal arbors of fibers with receptive fields within a 20^{o} area are recorded at a single tectal site.

For several weeks after optic nerve transection, no optic fiber activity can be detected in the tectum. Thereafter, units with normal optic nerve receptive field properties re-appear. At first, only very low amplitude spikes can be recorded from these optic fiber arborizations. Most units are found at inappropriate tectal sites, and receptive fields mapped in single electrode penetrations often are widely scattered. Topographic reorganization along the mediolateral tectal axis lags behind rostrocaudal reorganization; thus, many units reach the correct rostrocaudal distance while they are still too far medial or lateral.

To determine whether there is mediolateral disorder not only of axon arbors in the tectum but also of axon pathways in the regenerating optic tract, the receptive fields of arbors recorded in the tectum were mapped after cutting part of the tract. In normal frogs, a cut extending from the midline partway across the tract at the rostral margin of the tectum severs optic axons with receptive fields in the temporo-superior quadrant of the visual field. During regeneration, however, a similar cut spares many units with temporo-superior fields. This result implies that some fibers which normally enter the tectum via the most medial parts of the optic tract regenerate through other parts of the tract. This mediolateral disorder of axons in the tract may contribute to the slow course of mediolateral reorganization of their arborizations in the tectum.

1214 ELECTRON MICROSCOPIC OBSERVATIONS OF OCCIPITAL CORTEX IN DIFFERENTIALLY REARED RATS. Roger N. Walsh, Dept. Psychiatry, Stanford Medical School, Stanford, Ca. 94305, and Robert A. Cummins, Dept. Psychology, University of West. Australia.

Animals reared in sensorilly enriched or deprived environments exhibit differences in brain anatomy and chemistry. Among the many changes that have been reported, light microscopy has revealed that the enriched animals have thicker cerebral cortices which contain neurons with increased dendritic branching and numbers of dendritic spines. There is considerable theoretical interest in the nature of possible synaptic changes but as yet there have been only two electron microscopic studies. These reported only an increased synaptic size rather than the expected increase in synaptic numbers. The present studies aimed at investigating this apparent discrepancy as well as examining neuronal and neuroglial changes.

Littermate pairs of male Wistar rat pups were differentially reared for 30 days from weaning. The first group were isolated and deprived of sensory stimulation while the second were housed together in large open mesh cages and supplied with a new selection of toys each day (enriched group). Sections of the occipital cortex were photographed at 14K and the negatives were then projected onto a screen where the number of synapses were counted and the length of the postsynaptic thickening was measured. A total of 1800 synapses were sampled. The numerical density of synapses was greater in the enriched brains (18.5%, $P<0.05$), although average synaptic length was actually greater in the isolated cortex (6.1%, $P<0.05$). It would appear that a de novo growth of synapses has occurred in all layers of the cortex of animals exposed to enrichment and that these new synapses are of small size and therefore reduce the average synaptic size below that of their isolated counterparts.

1215 **WITHDRAWN BY AUTHOR**

1216 PROTEIN SYNTHETIC RESPONSE OF THE RAT SPINAL CORD TO HEMISECTION AND PUROMYCIN IMPLANTS. M.R. Wells,* J. J. Bernstein and M.E. Bernstein. Dept. Neurosci., Coll. Med., Univ. of Fla., Gainesville, Fla. 32610

Recent studies of the morphological response of the rat spinal cord to hemisection indicated that varicosity formation on the dendrites of ventral horn motoneurons may be suppressed (for a least 60 days) by puromycin. However, the neuroglial reaction was normal. The present study investigates the protein synthetic reponse of the rat spinal cord to a left hemisection at T_2 and puromycin (1mM) gelfoam implants into the lesion site as measured by the incorporation of ^{3}H-lysine into the trichloroacetic acid (TCA) precipitable protein one hour after a 200 µCi subcutaneous injection. Measurements were made 6, 12, and 24 hours, 3, 7, and 14 days later. Groups consisted of hemisection only, hemisection with gelfoam implants containing saline, hemisection with gelfoam and 1.0 mM puromycin in saline, sham operated and normal animals. Samples were taken from the left and right sides. There was a significant ($P < .05$) increase in the ^{3}H-lysine incorporation into protein in the area of the lesion of hemisected animals without puromycin treatment originating between 12 and 24 hours which increased over time compared to normal and sham operates. Puromycin treated animals exhibited a slight depression of ^{3}H-lysine incorporation into protein 6 hrs after hemisection which returned to near hemisected rates 12 hrs postoperative. In 7-14 day puromycin animals, residual effects were noted which did not seem to be associated with the direct protein synthesis inhibiting capacity of the puromycin. These data suggest that any direct effects of puromycin which results in neuronal resistance to varicosity formation for 60 days probably occurs within the first six hours after hemisection and implantation. Supported by NS06164.

1217 FRONTAL DECORTICATION FACILITATES HYPOTHALAMIC SELF-STIMULATION DURING FOOD DEPRIVATION. J. Roger Wilson. Dept. Psychiatry, MHRI, Univ. of Michigan, Med. Sch., Ann Arbor, MI. 48109.

Recent evidence indicates that frontal decortication and food deprivation lower sensitivity thresholds to neurochemically-mediated skeletal activity and feeding patterns. Attempts to assess an interaction between these variables and reinforcing hypothalamic stimulation (ESB) have been hampered by the technical inability of maintaining patent electrode/cannula assemblies in the decorticate preparation. The research described here uses a successful technique (Wilson, Vardaris & Schweikert, P & B, 14, 875, 1975; Wilson & Vardaris, P & B, 9, 809, 1972) to determine whether frontal ablations alone, or in combination with 24, 48, and 72 hr of food deprivation, influence the ESB sensitivity thresholds. Self-stimulation performance was examined in 16 adult, male, albino rats in a shuttlebox situation. Half (8) of the animals were given bilateral frontal ablations by subpial aspiration and allowed one wk postoperative recovery before testing. The remaining animals underwent sham operations. The ESB was delivered when the animal crossed over a small partition into either chamber. Shuttle latencies were measured as a function of deprivation condition. Stimulation from bipolar electrodes consisted of a single, 1500 msec train of square wave pulses, pulse duration of 8 msec, 100 PPS, at a peak current of 100 μA. Results showed that decortication lowered baseline threshold for ESB and facilitated threshold alterations following food deprivation. Both effects were independent of general activity changes. The implications of these and related observations will be discussed in terms of current notions that frontal lesions interrupt common neurochemical pathways underlying feeding and reinforcement and that time-dependent "denervation supersensitivity" occurs.

1218 CYTOPLASMIC LAMINATED BODIES IN THE LATERAL GENICULATE NUCLEUS OF NORMAL AND DARK REARED CATS. I.G. Worden and R.E. Kalil. Dept. of Anat., Univ. of Wis., Madison, WI 53706

When cats are reared in the dark from birth, cells in the lateral geniculate nucleus (LGN) fail to grow at a normal rate and subsequently atrophy (Kalil, Anat. Rec. 181, 1975). In addition, dark rearing also produces an increase in the number of cytoplasmic laminated bodies (CLBs) in the LGN (Kalil, Neuroscience Abstracts, 1, 1975). We have extended this latter observation by counting the number of CLBs in lamina A of the LGN in 3 groups of cats: (a) normal, light reared (LR) animals, ages 3 wks. to adult; (b) cats dark reared (DR) from birth until 3 to 16 wks.; and (c) DR-LR cats (DR from birth to 12 wks., then LR for 4 to 9 months). Since most CLBs are located eccentrically in the cell body and not in the plane of the nucleolus, we have measured CLBs/mm^2 of lamina A. In LR cats few CLBs are seen until 8 wks. At this age and thereafter the number of CLBs remains fixed at approximately 0.7/mm^2. No difference is evident between LR cats and DR-LR cats of comparable age. In contrast a threefold increase in CLBs/mm^2 occurs in the LGN of 12 and 16 wk. DR cats. One micron serial section reconstructions of DR geniculate cells suggest that this increase is not due to a proliferation of CLBs within individual neurons since multiple CLBs are not found. Although the overall mean area of LGN cells in LR and DR-LR cats is larger (ave. 320μ^2) than in DR cats (ave. 230μ^2), CLBs in all 3 groups are found only in cells (ave. 180μ^2) which are amongst the smallest in the LGN.

1219 RETINOFUGAL PATHWAYS IN TECTUMLESS GOLDFISH. Dean Yager and S. C. Sharma. S.U.N.Y. College of Optometry and Department of Ophthalmology, New York Medical College.

In goldfish, the retinal projection is completely crossed, and the majority of optic fibers terminate in different layers of the contralateral optic tectum. However, various thalamic nuclei - nucleus rotundus, nucleus dorsolateral thalami, area ventralis lateralis of the thalamus, L.G.N., area pretectalis, area corticalis - and the magnocellular nucleus of the hypothalamus also receive direct projections from the contralateral eye.[1]

In the present study, the optic tectum was completely removed bilaterally under anesthesia, and the animals were allowed to survive up to a year. One eye was injected with 10 microcuries of tritiated proline and the brain was examined autoradiographically. The thalamic visual nuclei were labelled more heavily than in control animals. Moreover, the contralateral tori semicircularis, nucleus isthmi, ventrolateral tegmental areas, and part of the reticular formation were also labelled heavily; these latter areas receive no direct retinal projection in the normal fish.

A number of behavioral tests of visual function were also made on control and experimental animals.

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[1]Sharma, S. C. Brain Research, 39, (1972), 213.

1220 FORMATION AND SUBSEQUENT SUPPRESSION OF INCORRECT SYNAPSES DURING SALAMANDER LIMB REGENERATION. J. W. Yip* and M. J. Dennis* (SPON: Juan Korenbrot). Depts. Physiol. Biochem., School of Medicine, University of California, San Francisco, CA. 94143.

In adult salamanders a foreign nerve will form functional synapses on denervated muscle fibers, but these are suppressed upon reinnervation by the correct nerve (Yip & Dennis, Nature 260, 350. 1976). We have tested the possibility that this ability to distinguish correct from incorrect innervation is put to use during limb regeneration. Intracellular recordings were made from muscle fibers in regenerating forearm flexor muscles, while independently stimulating the flexor and extensor nerves. At early stages of regeneration 27 of 64 fibers (5 muscles) were found to receive inappropriate synatic inputs from the extensor nerve. Some of these flexor muscle fibers were innervated by both flexor and extensor nerves. At later stages of development we found no evidence of synaptic input from the extensor nerve (191 fibers from 9 muscles examined). We conclude that during limb regeneration inappropriate neuromuscular synapses are sometimes formed but are subsequently removed in favor of the correct ones. (Supported in part by a training grant from the Sloan Foundation and a grant from the National Institutes of Health.)

1221 EFFECTS OF CHRONIC INSULIN ON BRAIN TYROSINE HYDROXYLASE AND GLUCOPRIVIC FEEDING IN 6-HYDROXYDOPAMINE-TREATED RATS. Michael J. Zigmond, Conrad D. Volz*, Edward M. Stricker, and Mark I. Friedman*. Departments of Life Sciences and Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

Rats given 6-hydroxydopamine (2 x 200 µg, ivt.) and who show 76% depletion of dopamine and 98% depletion of norepinephrine in telencephalon do not demonstrate the usual increase in food intake following inhibition of glucose utilization by 2-deoxy-D-glucose (500 mg/kg, i.p.). They do, however, increase food intake following 24-hr food deprivation or the administration of a more mild glucoprivic stimulus, such as protamine zinc insulin (4 units/day, s.c.). Thus, after 6-hydroxydopamine, central adrenergic function may be sufficient to sustain behavior during conditions of mild need but inadequate when demands are more severe (Stricker et al., Science, 189: 895, 1975). We now report that the administration of protamine zinc insulin (1-8 units/day) for 16 days resulted in a 213% increase in the apparent Vmax for tyrosine hydroxylase in hippocampus and an increase in hippocampal norepinephrine levels to 67% of control. These effects were accompanied by a restoration of the normal feeding response to 2-deoxy-D-glucose. Upon termination of insulin treatment, neurochemical and behavioral changes gradually disappeared over 6-12 weeks. These observations suggest that chronic insulin treatment can increase tyrosine hydroxylase activity in residual noradrenergic nerve terminals and thereby permit lesioned rats to respond appropriately to intense homeostatic challenges. (Supported, in part, by USPHS Grants MH-20620, MH-25341, and MH-00058.)

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1222 TAIL-PINCH STRESS REVERSES AMPHETAMINE ANOREXIA. S. M. Antelman*, A. R. Caggiula*, D. J. Edwards* and N. E. Rowland* (SPON: T.-M. Shih) Western Psychiatric Inst. & Clinic, Dept. Psychiatry, Univ. Pittsburgh, Sch. Med., & Psychobiology Program, Univ. Pittsburgh, Pittsburgh, Pa. 15260.

Electrical stimulation of the lateral hypothalamus (ESLH) has been reported to counteract the anorectic effects of amphetamine (Thode & Carlisle, J. Comp. Physiol. Psychol., 1968, 66, 547-48). Since our own research indicates that feeding induced by tail-pinch stress resembles ESLH-induced feeding in many respects, we tested the extent to which tail-pinch could similarly overcome amphetamine anorexia. D-amphetamine sulfate (or its saline vehicle) was administered in doses of 0.5, 1.0 & 2.0 mg/kg (of the salt) to 22-hr-deprived male albino rats. A 15 min. ad lib feeding test was conducted one-half hr. after injection and this was immediately followed by a 10 min. tail-pinch test. This same procedure was also followed for the amphetamine congener, methylphenidate (5.6 mg/kg). At the doses used both drugs caused severe anorexia during the ad lib (no tail pinch) tests, with amphetamine producing dose dependent effects ranging from 67% to virtually 100% suppression of feeding relative to vehicle-treated controls.

In stark contrast to these results, however, were the findings obtained during the tail pinch period. In every instance, the tail pinch stress completely reversed the anorectic influence of amphetamine and methylphenidate.

A number of studies have suggested that the anorectic effects of amphetamine may be due to its action in releasing and thereby indirectly overstimulating dopamine (DA) receptors. Moreover, we (Antelman, et al., Brain Res., 1975, 99, 319-337) have previously demonstrated that tail-pinch-induced eating appears to depend on the nigrostriatal DA system. Therefore, we sought to determine whether tail-pinch might modify the effects of amphetamine on DA release in the striatum. In order to do this, we measured striatal levels of the DA metabolites, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) following amphetamine (1.0 mg/kg) and/or tail-pinch. The method used for these determinations was the sensitive gas chromatographic procedure of Watson, et al., Life Sciences, 1974, 15, 2167-78. The results of our assays indicated no change in HVA following either amphetamine or tail-pinch. DOPAC, on the other hand, declined significantly following both amphetamine and tail-pinch. When these stimuli were combined, there was a slight, though not significant attenuation of the effects of amphetamine on DOPAC levels.

Although the mechanism by which tail-pinch reverses amphetamine anorexia remains to be elucidated, the phenomenon itself is intriguing and of potential clinical importance. Thus, should further research indicate that tail-pinch stress can also counteract the anorectic actions of other, clinically approved agents, this would raise serious questions regarding the efficacy of such compounds in dealing with stress-related hyperphagia in humans.

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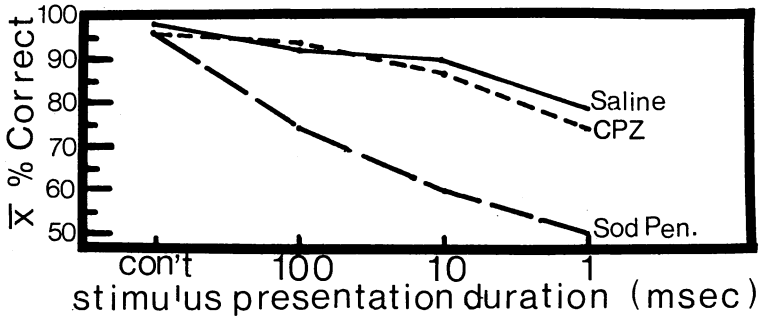
1223 VISUAL INFORMATION PROCESSING IN RHESUS MONKEYS: EFFECTS OF SODIUM PENTOBARBITAL AND CHLORPROMAZINE. Raymond T. Bartus and H. R. Johnson*, Psychopharmacology, Parke-Davis Res. Labs, Ann Arbor, MI. 48106.

Although chemically quite different, barbiturates (such as sodium pentobarbital) and certain phenothiazine derivatives (such as chlorpromazine) are both known to markedly depress the function of the reticular formation. The reticular formation, in turn, plays an important role in behavioral functions involving general arousal, alerting and selective attention. It is therefore not too surprising that behaviorally, both classes of drugs produce profound sedative effects as well as deficits on tasks which measure attentional mechanisms. However, the exact nature of their specific effects, and therefore their underlying mode of action on the reticular formation may be quite different. For example, on the basis of currently available data, it has recently been suggested that while chlorpromazine (CPZ) intermittently reduces sensory input, so that information is not appropriately transferred to motor effector systems, barbiturates impair performance by retarding the rate at which sensory stimuli can be processed and integrated (Pragay & Mirsky, Psychopharmacologia (Berl.), 28, 73-85, 1973; Mirsky, et al., Psychopharmacologia (Berl.), 41, 35-41, 1975).

Because direct support for this differentiation is lacking, the present study was designed to evaluate the degree to which sodium pentobarbital and CPZ differentially reduce the speed or efficiency with which sensory information can be processed and assimilated. Basically, this was accomplished by pre-training 5 Rhesus monkeys on a visual discrimination problem and then empirically determining, for each monkey, the highest dose of sodium pentobarbital and CPZ under which no impairment occurred. (This provided individualized doses where confounding effects on mechanisms involved with simple visual acuity, motivation, psychomotor co-ordination, arousal, etc. would be negligible.) Once this dose was established, each monkey was tested with varying durations of tachistoscopically presented stimulus information. If either sodium pentobarbital or CPZ selectively impair the speed with which sensory stimuli can be processed and/or integrated, differential effects of that drug should be seen on discrmination performance as the stimulus presentation becomes increasingly more brief.

The results of this study (see Fig.) clearly demonstrate that when continuous stimulus information is available, no effects of the two drugs are observed at the doses used, but that as the duration of stimulus presentation is decreased, the effects of sodium pentobarbital become more severe relative to saline control and CPZ ($p < .005$). Since CPZ did not differ from the saline control at any presentation duration, it can be concluded that only the barbiturates selectively influence the rate at which sensory information can be processed and assimilated. These data therefore support the notion that although both classes of drugs apparently have a direct influence on reticular formation activity, their effects on attention (and therefore their presumed mode of action) appear to be qualitatively different.



1224 EFFECTS OF IMIPRAMINE AND MESCALINE ON THE IN VIVO METABOLISM OF ^{3}H-L-TRYPTOPHAN IN THE CEREBROLATERAL VENTRICLES OF RATS. L. P. Dwoskin*, H. A. Tilson*, K. M. Kantak*, J. M. Stein*, and M. J. Wayner. Brain Res. Lab., Syracuse University, Syracuse, NY 13210.

Rats were implanted with push-pull cannula in the right cerebrolateral ventricle and were perfused at a constant rate of 37.0±0.5 µl/min for 75 min. The freshly prepared perfusate contained 1 µCi of generally labelled ^{3}H-L-tryptophan per ml of artificial cerebrospinal fluid. After the collection of eight 5 min samples the rats were randomly injected with either 0.9% saline, 5 or 15 mg/kg of imipramine hydrochloride, or 15 mg/kg of mescaline hydrochloride. For one experimental condition no injection was given. Perfusate collected 10-20 min prior to and 20-40 min following injection was analysed by bidirectional thin layer chromatography and liquid scintillation spectroscopy. Analysis of the total radioactivity in the 15 successive samples indicated an asymptotic number of counts beginning with the collection of the fifth sample. The same absolute amount of ^{3}H-radioactivity was detected in the perfusate of drug injected and saline or no injection controls. The two control conditions did not differ in this or anyother respect and were combined in data analysis. Since the absolute amounts of ^{3}H-5-hydroxytryptamine (^{3}H-5HT) and ^{3}H-5-hydroxyindoleacetic acid (^{3}H-5HIAA) formed from ^{3}H-tryptophan tended to vary between perfusions, the data are expressed as the nCi of ^{3}H-radioactivity of 5HT or 5HIAA per µCi of total ^{3}H-radioactivity measured. The amounts of ^{3}H-5HT and ^{3}H-5HIAA formed per µCi of total ^{3}H-radioactivity for the samples collected 10-20 min prior to injection was relatively constant for the 3 drug treatments and combined control conditions. The IP injections of mescaline and imipramine produced significant alterations in the appearance of ^{3}H-5HT and ^{3}H-5HIAA in the ventricular perfusate. Analysis of successive 5 min samples of perfusate taken 20-40 min following the 15 mg/kg mescaline injection showed statistically significant decreases in ^{3}H-5HT in 2 of the 5 samples and decreases in ^{3}H-5HIAA in only 1 of the 5 samples. Imipramine, on the other hand, significantly increased the amounts of ^{3}H-5HT in 2 of the 5 samples at 5 mg/kg and 4 of the 5 samples at 15 mg/kg. Imipramine tended to decrease ^{3}H-5HIAA but significant decreases were observed following the 15 mg/kg dose only.

1225 SELF-STIMULATION: SITE-SPECIFIC TOLERANCE TO CHRONIC DOPAMINE RECEPTOR BLOCKADE. Alan J. Eichler*, Seymour M. Antelman* and Alan E. Fisher* (SPON: K.R. Carlson). Psychobiology Program, Dept. Psych., Univ. Pittsburgh, Pittsburgh, Pa. 15260

The use of neuroleptics to study dopaminergic (DA) and noradrenergic (NE) components of self-stimulation (ICSS) from various brain sites has to date involved only acute drug administration. We report here the effects of chronic neuroleptic treatment on ICSS behavior. This approach allows the study of ICSS during stages of tolerance and withdrawal, as well as acute depression. Rats were trained to self-stimulate in one of the following three sites: (1) pars compacta of the substantia nigra (A-9), (2) "far" lateral hypothalamus (FLH; region of DA fibers of passage), (3) "near" lateral hypothalamus (NLH; region of NE fibers). After baseline was obtained, each animal was injected daily for two weeks with the DA receptor blocker spiroperidol. Testing continued for 30 days following the injection period.

ICSS was initially abolished at all three sites using a 60ug/kg dose of spiroperidol. However, both the A-9 and FLH placements began exhibiting tolerance 5-7 days into the injection period. Rates then gradually increased and reached approximately 80% of baseline by termination of drug treatment (14 days). During the withdrawal phase, both of these sites displayed a 50% rate increase over pre-injection baseline which was still evident 30 days later. Although no behavioral tolerance occurred to a 120ug/kg dose, a comparable 50% rate increase during withdrawal was observed. The similarity between pre- and post-drug extinction curves indicated that the withdrawal rate increase was not reflective of stereotyped behavior. NLH ICSS, on the other hand, evidenced no tolerance to daily injections of 30, 60 or 120ug/kg of spiroperidol. Rates remained depressed 70% for the 30ug/kg dose and 95% for 60 and 120ug/kg during the entire injection period. Upon withdrawal, rates returned to and stabilized at baseline levels.

These data point to a common neurochemical element between A-9 and FLH ICSS. Since fibers originating from DA cell bodies in A-9 course through the FLH, it is suggested here that dopaminergic pathways play an important role in ICSS from these two regions. Furthermore, the occurrence of tolerance and a withdrawal rate increase at the FLH, but not at the NLH, indicates a distinct behavioral differentiation between these two lateral hypothalamic sites. Whereas, acute neuroleptic administration depressed ICSS at all three tested placements, the chronic approach has permitted both a comparison and differentiation among ICSS sites. (Supported by Grants MH24114-S.M.A.-and MH1951-A.E.F.-)

1226 THE EFFECT OF MEPERIDINE ON BEHAVIORAL THERMOREGULATION IN THE MOUSE.
Peter K. Gessner and Carolyn C. Clarke*. Pharmacology Dept., State Univ. of N.Y. at Buffalo, Buffalo, N.Y. 14214.

We have previously reported that administration of meperidine (pethidine) to mice at low ambient temperatures leads to a marked hypothermia (J. Pharmacol. 189: 90, 1974) while its administration at a high ambient temperature leads to a significant hyperthermia (Fed. Proc. 35: 264, 1976). This study was undertaken to determine whether meperidine impaired thermoregulation by disruption of afferent or efferent information flow. Mice were placed singly in a chamber through which could be circulated air at either 15° or 45°C. The animals were trained to switch from one to the other air stream by pressing a lever. Integration of the amount of time spent by the animals in the two air streams during any one ten min. period gave the average chamber temperature chosen by mice during that period. Rectal temperatures were determined by a thermister probe inserted 2.5 cm into the rectum and taped to the tail. The animals were otherwise unrestrained. Observations were continued for 1 hour. Meperidine in a dose of 15 mg/kg i.p. led under these conditions to a maximal 77% decrease in response rates ($P < 0.05$), a maximal 4.7°C decrease in body temperature ($P < 0.01$) and a maximal 7.4°C decrease in chamber temperature ($P = 0.01$) all relative to those of control animals. Since a random decrease or cessation in operant behavior would not have been expected, a priori, to result in a net lowering of chamber temperatures, a second experiment was undertaken. In this experiment the paradigm was changed so that, while the animal was still able to alter chamber temperature as previously, it would be automatically presented with air at 45°C every 3 minutes unless it had been exposed to this air flow within the previous 0.4 min. Meperidine administered animals under these conditions also showed a maximal 77% decrease in response rates ($P < 0.02$) relative to those of controls. However, the non-randomness of this decrease was evident from the chamber temperatures maintained by the meperidine administered animals not rising above those maintained by the controls. In fact the meperidine administered animals maintained, on average, lower chamber temperatures than the controls though this difference was not a statistically significant one. They also exhibited a significantly lower body temperature than controls; this difference being maximally 1.8°C ($P < 0.01$). These results suggest that (1) meperidine in a dose of 15 mg/kg i.p. significantly depresses operant behavior but that (2) this does not compromise the animal's ability to behaviorally thermoregulate. On the other hand, meperidine in this dose appears to decrease differentially the animal's responsiveness to cold environmental stimuli suggesting a disturbance of afferent information flow.

1227 POTENTIATION OF STEREOTYPED BEHAVIOR AND LOCOMOTOR ACTIVITY WITH ANTIDEPRESSANTS: A DOPAMINERGIC EFFECT? Angelos E. Halaris and Jeffrey J. Feigenbaum*. Dept. Psychiatry, Univ. of Chicago, Chicago, Il. 60637

We have previously reported that psychotropic drugs (antidepressants, neuroleptics, stimulants) inhibit ^{3}H-dopamine (DA) uptake into nuclei-free homogenates from rat brain [Halaris et al., Biochem. Pharmacol. 24, 1896-1898 (1975); Halaris and Freedman, Res. Publ. Assoc. Res. Nerv. ment. Dis. 54, 247-258 (1975)]. Drug concentrations necessary to achieve 50% inhibition of uptake range from 0.1 - .8 μM depending on the brain region studied. Animals pretreated with antidepressants showed significant and time-dependent DA uptake inhibition. In confirmation of recent reports in the literature, we found that antidepressants and neuroleptics are also effective releasing agents of dopamine. To deduce physiological significance of the above in vitro effects, we undertook studies of locomotor activity (LA) and stereotyped behavior (SB). LA and SB are currently thought of as being regulated by central monoaminergic pathways. Animals were pretreated with either d-amphetamine (AM) (5 mg/kg, i.p.) or apomorphine (AP) (1 mg/kg, i.p.) and observed until marked attenuation of LA and SB was manifest (3.5 hr after AM; 45 min after AP). They were then injected with one of the following antidepressants: desipramine (DMI), amitriptyline (AMI), chlorimipramine (CMI), imipramine (IMI). Marked enhancement of LA was observed with all drugs tested following pretreatment with AM or AP and the order of potency was: DMI $\hat{}$ AMI > CMI > IMI. In the unpretreated animal, antidepressants induced LA characterized by intense paroxysmal bursts of hyperactivity interspersed with periods of total inertia. This effect was best observed with CMI. With this drug, a bimodal pattern of bursts of LA was noted, beginning immediately after the i.p. injection and lasting for 15 min; following a 15 min period of inactivity, a second LA peak was observed at 30-45 min post injection. If AM (1 mg/kg, i.p.) preceded CMI by 1 hr, an initial 30 min latency period was followed by the bimodal pattern of enhanced LA. Similar effects were observed with the other antidepressants.

SB was scored utilizing a modified rating scale (0-4) based on that described by Costall and Naylor (1972) and animals were observed through a one-way mirror to avoid the "freezing" effect. Following an initial 30 min habituation period, animals were continuously observed and scored every 15 min. None of the antidepressants tested at 25 mg/kg, i.p., elicited SB by themselves. None of the animals injected with AM or AP alone exhibited biting; 27% exhibited licking (high intensity components of SB). However, all antidepressants significantly potentiated the AM or AP-induced SB in that 50-75% of all animals displayed biting or licking. In addition to potentiating SB, DMI elicited gnashing in 62% of rats pretreated with AM and in 50% of animals pretreated with AP. With all antidepressants, following AP pretreatment the enhancement of SB was not nearly as great as that observed with AM pretreatment. The rank order of efficacy of the antidepressants tested in enhancing SB was as follows: DMI > AMI > CMI > IMI, whereby DMI and AMI were almost equipotent.

In view of the fact that norepinephrine (NE) is not related to the mode of action of AP, the obtained results--at least in the case of AP--indicate that DA is in all likelihood involved in the enhancement of LA and SB induced by antidepressants. There is considerable controversy at present over the possible role of NE in mediating LA or SB and it is possible that the presence of more than one transmitter is necessary to elicit the behavioral effects described above. Our data strongly suggest that DA may play a significant role in the mode of action of tricyclic antidepressants. (Supported by research grant 506-03 from the Illinois Department of Mental Health).

1228 ALTERATIONS IN REPRODUCTIVE BEHAVIOR PATTERNS OF FEMALE RATS FOLLOWING ADMINISTRATION OF CATECHOLAMINE-DEPLETING DRUGS. James G. Herndon, Jr.*, Anthony R. Caggiula*, Donna Sharp*, Diane Ellis*, Michael J. Zigmond, and Edward S. Redgate*. Psychobiology Program, Depts. of Psychology & Life Sciences, University of Pittsburgh, & Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pa. 15260 (SPON: E. Bruce Goldstein)

An enhancement in the lordosis component of sexual receptivity was obtained in ovariectomized, hormone treated female rats after both chronic and acute reduction of brain catecholamine (CA) activity. First, an intraventricular 6-hydroxydopamine (6-OHDA) treatment which produced 66% & 53% depletions of telencephalic norepinephrine and dopamine respectively, resulted in a moderate increase of lordosis frequency and intensity in females given low doses of estrogen and tested with vigorous males. Second, the enhanced responding which followed 6-OHDA could be markedly intensified by the addition of the CA synthesis inhibitor alpha-methyl-p-tyrosine (AMT) in a dose (100mg/kg i.p.) that had only a weak and inconsistent potentiating effect when given alone. Third, the combined 6-OHDA and AMT treatment greatly increased the duration of lordosis in estrogen/progesterone treated females in whom lordosis frequency and intensity were near maximal. Fourth, suppression of adrenal function, which was produced by dexamethasone and verified by radioimmunoassay of plasma corticosterone, did not prevent 6-OHDA enhancement of lordosis frequency, intensity or duration, suggesting that this phenomenon is not dependent on the release of adrenal progesterone. Finally, treatments which enhanced lordosis failed to increase soliciting behavior. These observations suggest a direct or indirect inhibitory influence of brain CA-containing systems on female sexual behavior. This effect appears to be on the immobility or "stop" component of receptivity, rather than on the entire copulatory pattern. (Supported by grants MH16581, MH20620, MH00058 and NIH04095).

1229 EFFECTS OF DI-N-PROPYLACETATE ON MOTOR PERFORMANCE AND DISCRIMINATION REVERSAL LEARNING IN THE RAT. Beverly M. Kulig. Dept. Physiol., Univ. of Leiden, Leiden, The Netherlands.

Studies were carried out to assess the effects of the antiepileptic di-n-propylacetate (DPA) on learned motor behavior and successive discrimination reversal learning in the rat. Rats were trained to a 99% criterion level to avoid electric shock by running treadmill fashion on a continuous motor-driven belt, and the effects of saline and 100, 200, and 400 mg/kg DPA were measured in three 2-min trials 15 min after i.p. administration. Regression analysis revealed a significant correlation between performance and DPA blood levels when DPA levels ranged from 100 to 500 mg%. Also, rats showed a dose-dependent impairment of performance following DPA while saline was without effect. Moreover, animals receiving 100 and 200 mg/kg showed a progressive improvement over the three trials (acute functional tolerance) in contrast to the 400 mg/kg group which showed no progressive improvement. Acute functional tolerance was further investigated in two groups of rats which received three trials either 5 or 20 min later. Analysis of variance revealed a progressive improvement over trials for both groups, however, the groups did not differ with respect to initial impairment or rate of improvement. These studies indicate that the moving belt test is a sensitive method for assessing the neurotoxic effects of antiepileptics, that behavioral augmentation of tolerance to DPA can occur with dose levels less than 400 mg/kg, and that increased length of exposure to the drug on an acute basis does not alter the development of functional tolerance.

In addition, the effects of DPA at a dose reported to enhance acquisition of behavior (Misslin et al., Psychopharmacologia, 44:263, 1975) was investigated using successive discrimination reversal learning. Rats were trained to bar-press for sucrose reward in a go/no-go situation in the presence of light or tone. S+ and S- were reversed daily until stable reversal behavior was achieved and the effects of saline, d-amphetamine (0.5 mg/kg), and DPA (100 mg/kg) were measured. The mean percentage of errors for the amphetamine-treated animals was significantly lower in the drug state while saline and DPA treatments were without effect. Analysis of performance over blocks of trials revealed no difference between the groups on initial trials, however, amphetamine-treated animals significantly improved during the test session. Taken together, these experiments indicate that DPA at dose levels well below the anticonvulsant ED50 interferes with learned motor behavior and does not improve discrimination performance maintained by positive reinforcement.

1230 SEX DIFFERENCES IN BEHAVIOR FOLLOWING REARING ON A HIGH-CARBOHYDRATE DIET. Barbara J. Morley*, Steven L. Cohen*,and Alex Poplawsky*. (Spon: E. Gfeller). Neurosci. Prog., UAB Med. Sch., Birmingham, AL. 35294 and Dept. Psych., Bloomsburg St. Coll., Bloomsburg, Pa. 17815

It has recently been established that "normal" variations in diet are correlated with levels of brain neurotransmitters and alterations in behavior. One neurotransmitter which has long thought to be influenced by diet is serotonin. It has been demonstrated, for example, that a tryptophan-poor diet results in lowered levels of brain serotonin and decreased pain threshold and that rehabilitation with tryptophan restores both pain threshold and serotonin level (Lytle, Fed. Proc. 429, 1975). Wurtman & Fernstrom (Am. J. Clin. Nutr. 28: 638, 1975) have also demonstrated altered brain serotonin in levels by the acute feeding of a high-carbohydrate diet.

Although it is known that serotonin is involved in a number of behaviors, little is known about variations in behavior resulting from feeding on a high-carbohydrate diet.

We now report that female rats raised from weaning on a high-carbohydrate diet (ICN Biochemicals) have lower activity scores and disrupted active shuttle-box acquisition in comparison with females raised on a standard laboratory diet. In contrast males were not differentially affected by such a diet. Statistical analysis was performed using a repeated measures analysis of variance. Although females were found to have higher activity scores and avoidance scores, the sex x diet interaction was significant for both behaviors. No statistically significant differences were found in avoidance latencies, escpae latencies, or intertrial crossing among any of the groups. Heart rates were also taken. Females were observed to have higher heart rates but the sex x diet interaction was not significant.

These data show that chronic feeding on a high-carbohydrate diet can affect behavior and are consistent with the findings that brain neurotransmitter levels and behavior are partly determined by diet.

1231 MECHANISM OF LITHIUM TRANSPORT IN RED BLOOD CELLS. G.N. Pandey, J.I.Javaid, J.M.Davis,D.C. Tosteson. Dept. of Psych., Pharma. & Physiol. Sci., Univ. of Chicago and Ill. State Psychiatric Inst., Chicago, Ill.

Lithium salts are useful in the treatment of manic states and in the treatment of a subgroup of depressed patients. It has been reported that the ratio of the Li^+ concentration in red blood cells (RBC) to the Li^+ concentration in plasma is higher in patients who respond to Li^+ than in non-responders. This apparent abnormality in the distribution of lithium across the RBC membrane prompted us to investigate further the mechanism of Li^+transport in human RBC. We had earlier demonstrated the genetic control on Li^+ distribution across RBC membrane. We had also reported the presence in human RBC membranes of a Na^+ - Li^+ counter-flow system which is insensitive to ouabain but promotes net uphill transport of lithium driven by an oppositely directed electrochemical potential gradient for sodium ions. We now report three components of Li^+ influx. Washed cells were incubated in a buffer containing 40 mM LiCl, 100 mM NaCl, 5 mM glucose and 10 mM glycyl glycine pH 7.4 at 37°C and Li^+ in RBC determined in samples taken from time to time. Li^+influx is inhibited additively by both ouabain and phloretin. Ouabain-sensitive Li^+influx is inhibited completely by external K^+ and partially by external Na^+. Phloretin-sensitive Li^+influx is inhibited by external Na^+but not by external K^+ . Phloretin but not ouabain inhibited the Li^+ - Na^+ counter-flow system. These data clearly indicate three different pathways for Li^+ influx. These pathways may be mediated by different membrane molecules but also may involve different parts and/or conformation of the same membrane molecules. The ouabain sensitive pathway for Li^+ influx is probably the same as that used by K^+ in the normal operation of Na^+ - K^+ pump. Lithium distribution between human red cells and plasma in vivo seems to depend mainly on the quantitative balance between phloretin-sensitive Li^+ - Na^+ counter-flow and phloretin-insensitive, ouabain-insensitive Li^+ transport. It is now important to determine which of these moieties of Li^+ transport is abnormal in patients with affective disorders.

1232 BEHAVIORAL AND BIOCHEMICAL EVIDENCE FOR SUPERSENSITIVITY IN RATS AFTER WITHDRAWAL FROM CHRONIC HALOPERIDOL, CLOZAPINE AND THIORIDAZINE. Robert C. Smith, N. Narasimhachari, Carol Tamminga and John M. Davis, Ill. State Psychiatric Inst., Chicago; and Tex. Res. Inst. Ment. Sci., Houston, Texas 77025.

Previous reports from our group (Psychopharm. Comm. 1:273, 1975) or other researchers have indicated that after cessation of chronic treatment with haloperidol (HAL) or chlorpromazine, but not clozapine (CLZ) (Psychopharmacologia 41:97, 1975), rats exhibit behavioral supersensitivity to dopamine agonists as indicated by increases in stereotyped and turning behaviors. We now report that rats withdrawn from a 6-8 weeks course of daily treatment with a higher dose of CLZ (25 mg/kg), or Thioridazine (THOR) 20 mg/kg, as well as several doses of HAL (1 mg/kg - 5 mg/rat), all exhibited significantly greater stereotyped behavior after 1 mg/kg apomorphine than their saline (SAL) controls. This supersensitive response peaked 5-7 days after cessation of neuroleptics, but a similar trend was evident up to 3 weeks. One biochemical indicator of receptor supersensitivity to dopamine agonists, greater decreases in dopamine turnover after APO, was assessed by measuring HVA in rat caudate after administration of probenecid + APO 1 mg/kg. HVA was quantitatively determined by selective ion monitoring with the specific gas-chromatographic mass spectrometric method previously described (Clin. Chem. Acta 50:337, 1974; 62:245, 1975). Seven days after their last dose of neuroleptics, rats previously given HAL-5 mg or CLZ-25 mg/kg had lower HVA levels after APO than SAL controls (mean caudate HVA, ng/gm: HAL = 360, CLZ = 591; SAL = 985). Another possible biochemical indicator of supersensitivity, serum prolactin responses to APO, and also the effects of chronic neuroleptics on synaptosomal dopamine reuptake, are being investigated. Our results are consistent with the suggestion that chronic administration of CLZ, THOR, and HAL to rats may lead to a functional supersensitivity of post and/or pre-synaptic dopamine receptors. (Supported by FFRP 73-578 and FFRP 74-592.)

1233 CHRONIC BUT NOT ACUTE CHLORPROMAZINE ATTENUATES DISRUPTIVE EFFECTS OF N, N-DIMETHYLTRYPTAMINE (DMT) ON SHUTTLEBOX AVOIDANCE IN RATS. Stoff, D.M., Moja, E.A.*, Gillin, J.C., Wyatt, R.J. Laboratory of Clinical Psychopharmacology, SMR, IRP, NIMH, St. Eliz. Hosp., Wash., D. C. 20032.

The present data represents the first report that behavioral effects of DMT can be attenuated by neuroleptic pretreatment.

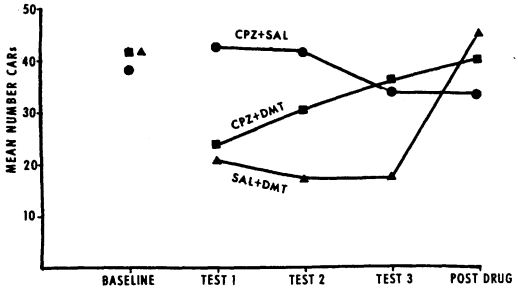
DMT, an endogenous indolealkylamine, may be involved in the pathogenesis of schizophrenia because it is a short-acting hallucinogen, and may be differentially elevated in schizophrenic patients. We recently reported dose-dependent DMT-disruption in trained rats on a conditioned avoidance response (CAR) in the shuttlebox; the threshold dose and time course of these effects correspond closely to DMT's psychological disturbance in humans.

Acute Neuroleptic Effects: Fischer 344/Mai rats (male, 200-300g) were trained in the shuttlebox to a baseline level of CAR criteria which was high and stable (≥80% CARs on 2 consecutive 100-trial sessions and a difference of ≤10% CARs on these 2 sessions) and tested after a 7-day rest according to a repeated measures design under 5 treatments: Saline + DMT 4.0 mg/kg (SAL+DMT, n=17), Chlorpromazine 0.5 mg/kg + Saline (CPZ+SAL, n=15), CPZ 0.5 mg/kg + DMT 4.0 mg/kg (CPZ+DMT, n=13), Haloperidol 0.05 mg/kg + Saline (HAL+SAL, n=15), and HAL 0.05 mg/kg + DMT 4.0 mg/kg, (HAL+DMT, n=10). The interval between the 2 injs (ip) was 28 mins and the rat was tested 2 mins after the 2nd inj.

Because the disruptive effect of DMT was restricted to the 1st 50 trials, CAR data was analyzed only during this period. One-way ANOVAR with repeated measures on CAR rate among baseline mean and 5 treatments showed significant drug effect ($F=15.86$, $p<0.01$). Post hoc tests: i. Expected disruption for SAL+DMT, relative to baseline ($p<0.01$); ii. no effect for CPZ+SAL or HAL+SAL, relative to baseline (ns); iii. no protection of disruption for CPZ+DMT, relative to SAL+DMT (ns); and iv. attenuation of disruption for HAL+DMT, relative to SAL+DMT ($p<0.01$).

Chronic Neuroleptic Effects: 15 rats were trained to same CAR criteria as above. There were 29 more consecutive sessions of 100-trials/day and after each session rats were injected ip with CPZ 1.0 mg/kg. Following 14 CPZ injs (Drug Test Day 1), 21 injs (Drug Test Day 2), and 29 injs (Drug Test Day 3), 5 of the 15 rats received SAL+DMT 4.0 mg/kg before the session, 5 received CPZ 0.5 mg/kg+SAL and 5 received CPZ 0.5 mg/kg+DMT 4.0 mg/kg; 24 hr after last inj there was a SAL retest (Postdrug Day).

For each drug group, separate one-way ANOVARs with repeated measures on CAR rate for sessions except Drug Test Days showed that CAR rate remained stable at baseline level ($Fs<1$). Similar analyses on the difference in CAR rate between mean baseline and Drug Days 1,2, and 3, showed a significant effect over time for CPZ+DMT ($F=7.82$, $p<0.05$) but no effects for CPZ+SAL or SAL+DMT ($Fs<1$). Relative to baseline, the Figure shows that over time (with increasing injs of CPZ): i. CPZ+DMT had progressive diminution in disruption; ii. SAL+DMT had about the same degree of disruption; and iii. CPZ+SAL remained at baseline CAR rate; CAR rate on Postdrug Day was same as baseline.



Thus, DMT disruption was attenuated by acute HAL, and to a degree directly related to length of CPZ pretreatment, consistent with stronger clinical potency for haloperidol and efficacy of long term neuroleptic drug treatment. These pharmacological parallels satisfy criteria for animal models of psychosis.

1234 TOTAL AND FREE PLASMA TRYPTOPHAN IN PATIENTS WITH AFFECTIVE DISORDERS.
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Recent data suggests a dependence of brain serotonergic function on free plasma tryptophan (FPT) concentrations. FPT has been reported to be reduced in female depressed patients compared to controls in one study (Coppen et al. Lancet 2:60, 1973) while others have found no differences or elevations in FPT (Peet et al. Brit. J. Psychiat. 128:255, 1976, Niskanen et al. Brit. J. Psychiat. 128:67, 1976). The present study examined the relationship of total plasma tryptophan (TPT) and FPT concentrations to affective illness in normal individuals and in patients with a diagnosis of primary affective disorder. A perturbation technique, employing the peripheral decarboxylase inhibitor carbidopa (L-hydrazino-α-methyldopa), was used to aid in elucidating the disposition and flux of plasma tryptophan.

Subjects (n=10; mean age 38.7 ± 4.1 years; 6 male and 4 female) with a diagnosis of primary affective disorder (8 bipolar, 2 unipolar) and normal controls (n=10; mean age 31.9 ± 2.4 years; 6 male and 4 female) were studied over a 10 day period. During this time, diet and activity were controlled. All subjects were drug free for at least 7 days prior to and throughout the duration of the study. FPT and TPT concentrations were determined on the 1st and 2nd day of a 3 day baseline period. Following completion of the baseline period, all subjects received carbidopa (100 mg t.i.d. p.o.) for the remaining days of the study. FPT and TPT measurements were again repeated on the last two days of the study period.

During the baseline period patients and controls did not show significantly different TPT (11.5 ± 0.47 vs. 12.1 ± 0.53, data expressed as mean ± S.E. μg/ml plasma) or FPT (1.87 ± 0.067 vs. 2.05 ± 0.098), although these values tended to be lower in patients. On the final two days of carbidopa administration TPT was not different between the two groups. FPT, however, was elevated in the patient group compared to controls (2.06 ± 0.069 vs. 1.78 ± 0.076; $p<0.05$). Significant differences in the disposition of plasma tryptophan were evident between patients and controls when comparing the carbidopa period to the baseline. For controls, TPT was unchanged, but FPT was reduced during the carbidopa period (1.78 ± 0.076) compared to the baseline period (2.05 ± 0.098; $p<0.05$). For the patients, TPT increased significantly on carbidopa (12.6 ± 0.55) compared to the baseline (11.5 ± 0.47; $p<0.05$). Similarly, FPT in the patients during the carbidopa period (2.06 ± 0.069) increased compared to the baseline levels (1.87 ± 0.067; $p<0.05$).

These results suggest that differences exist between normal individuals and patients with primary affective disorder with respect to the disposition and flux of tryptophan in peripheral plasma pools. The differences were not evident until the introduction of an agent which alters the flux of tryptophan through peripheral indoleamine synthesis. The results suggest the possibility of a deficit in tissue uptake of tryptophan in patients with primary affective disorder.

1235 THE FACILITORY EFFECTS OF DEXAMETHASONE ON THE DEVELOPMENT OF FUNCTIONAL TOLERANCE TO ETHANOL IN THE RAT. W. Gibson Wood. Dept. of Psychology and All-University Gerontology Center, Syracuse University, Syracuse, NY. 13210.

A number of studies have previously shown that chronic administration of ethanol results in a reduction in the activity of the hypothalamic-pituitary-adrenal cortex (H-P-AC) system. Also, it has been reported that strains of mice which show either a high or low preference for ethanol can be differentiated in their H-P-AC system response to ethanol. The high preference animals exhibit less H-P-AC activity (i.e., corticosterone)to ethanol than do the low preference animals.These strains also develop tolerance to ethanol at different rates. The high preference animals develop tolerance to ethanol at a faster rate when compared to the low preference animals. Thus, it would appear that there may be a relationship between the activity of the H-P-AC system and the development of tolerance to ethanol (Noble, 1971).

Until the present study, most experiments which have investigated the effects of ethanol on the H-P-AC system or on the development of tolerance have only been concerned with one or the other. On the other hand, studies which have examined the relationship between the H-P-AC system and tolerance have generally used procedures (e.g., removal of the adrenal glands) which are nonreversible, debilitating to the organism, and usually do not allow for the study of chronic tolerance development.

Therefore, the purpose of this study was to examine the proposed relationship between the development of tolerance to ethanol and the H-P-AC system, thru the use of a synthetic glucocorticoid, dexamethasone. It was predicted that animals receiving chronic injections of dexamethasone and ethanol would show a faster rate of tolerance development (this would be reflected in an increase in behavioral activity) to the depressant effect of ethanol than animals administered only ethanol.It also was the purpose of this study to determine whether animals chronically injected with ethanol and tested in an exploratory apparatus would develop tolerance at a faster rate compared to animals injected with ethanol but not tested until a later time. The results of the study indicated that administration of dexamethasone reduced ethanol-induced behavioral depression and accelerated the rate of tolerance development. These results were discussed in relation to a possible stimulant effect of dexamethasone on behavior, an increase in the metabolism of ethanol, and a possible accumulation of dexamethasone in the brain affecting an organism's response to ethanol.

It also was shown that animals injected with ethanol and tested,developed tolerance at a faster rate than animals injected but not tested until a later time. These results provided additional support for the theory of drug tolerance proposed by Kalant, LeBlanc, and Gibbins (1971).

1236 THE EFFECTS OF P-CHLOROAMPHETAMINE ON THE DEVELOPMENT OF BEHAVIOR AND BRAIN CATECHOLAMINES. Elizabeth Worsham*, Barbara J. Morley*, and Lynda Wallace*. (Spon: R. J. Bradley) Neurosci. Prog. UAB Med. Sch., Birmingham, AL, 35294, Ctr. For Alc. Stud., Rutgers Univ., Lyons, N. J. 07959, and Dept. Psych., Ramapo College, Mahwah, N.J.

Level of brain serotonin (5-HT) is known to be related to several behaviors in both animals and man, and experimental manipulations that deplete brain 5-HT also produce behavioral alterations. One method of altering brain 5-HT level is by the administration of p-chloroamphetamine (PCA) which produces long-term depletion of 5-HT but only traisient effect on norepinephrine (Sanders-Bush et al., J. Pharm. Exp. Ther. 192:33, 1975). Transient increases in locomotor activity have been demonstrated following PCA administration (e.g., Messing et al., Neuropharm., 15:157, 1976) and long-term effects on appetitive (Rosen & Freedman, Neuropharm. 14:585, 1974) and aversive instrumental conditioning.

In the studies reported here we were interested in the effects of PCA at early ages on behavior and brain neurochemistry in the developing and adult rat.

In experiment I, the effects of 7.5 mg/kg of PCA on activity, variable-interval (VI) rates, and the response to amphetamines were assessed in adult male albino rats. When PCA was administered one hour before the session, increased activity counts were obtained due to increased stereotyped behavior. On succeeding days, PCA-treated Ss had significantly suppressed activity scores in comparison with controls, but had significantly higher VI rates. Some evidence for potentiation of amphetamines was found using either behavior as the baseline. We further investigated whether the differences observed in operant rates were attributable to differences in activity in response to food deprivation. It was found that PCA-treated animals had higher activity scores when maintained at 80% of ad lib weight than when maintained ad lib. This effect was found long after animals recovered from body weight loss which accompanied the initial PCA injection.

In experiment II, male infant rats were injected with 7.5 mg/kg PCA at ages 3-5, 17, or 24-26 days of age. Juvenile and adult activity was assessed throughout development and the response to amphetamines was assessed in adulthood. VI rates and response to amphetamines was additionally studied in the 17-day injected animals. Animals injected at 3-5 days were found to have higher juvenile but not adult activity scores. Animals injected at 17 days had lower activity and VI rates. Animals injected at 26 days of age did not differ from controls in activity. Some evidence for potentiation was found in all groups

The results of studies in progress on the effects of PCA injections at these ages on 5-HT, 5-hydroxyindoleacetic acid, dopamine, and norepinephrine will also be presented. The long-term behavioral effects will be discussed in relationship to the effects of PCA on brain catecholamines.

1237 EFFECTS OF INTRACRANIALLY ADMINISTERED PENTYLENETETRAZOL, PICROTOXIN, AND GALLAMINE IN THE FREELY MOVING RAT. Bruce J. Albala* and Tibor Palfai*
Skytop laboratories, Dept Psych., Syracuse University, Syracuse, N.Y. 13210. (Spon: D. C. Goodman) Dept. Anat., Upstate Medical Center, Syracuse, N.Y. 13210.

Convulsions resulting from the intracranial administration of drugs is infrequently observed in the literature because of the use of anesthetized or restrained preparations. In this study, animals implanted with intraventricular cannulae were freely moving during the infusion of various doses of either pentylenetetrazol (PTZ), picrotoxin, or gallamine triethiodide at either one of three infusion rates (1 µl/min., 2 µl/min. and 5 µl/min. - for a total volume of 10 µl). The 2 % gallamine solution produced convulsions at the two highest infusion rates as reported for tubocurarine by Feldberg et al. (J. Physiol. 149:58, 1959). The 0.1 % picrotoxin and the 50 % PTZ solutions both produced circus movements and generally increased activity but only picrotoxin resulted in overt convulsions. Even at the highest (5 µl/min.) infusion rate animals which received PTZ did not convulse when injected intracranially with doses that were near the convulsive ED 50 dose given intravenously. Autoradiographic data on PTZ diffusion sites will also be presented.

1238 TEMPERATURE DEPENDENT EFFECTS OF HYPOXIA ON SELF STIMULATION RATES IN RATS. Zoltan Annau. Dept. Environm. Med., Johns Hopkins Univ., Balto., Md 21205.

Sixteen male hooded rats were implanted with stainless steel electrodes in the posterior lateral hypothalamus and trained to self stimulate. The animals were subsequently transferred to experimental chambers equipped with three levers. One lever activated a food dispenser, the second lever activated a water solenoid and the third lever delivered a 250ms 60Hz pulse train to the electrodes. All responses were on continuous reinforcement schedules. The chambers were maintained either at 21°C or at 32°C in a 12 hour light dark cycle at a constant air flow of 4 lpm. In a crossover design, 8 animals were first exposed to hypoxia at the high temperature first and the low second, and 8 animals were first exposed to low temperature. Currents on the electrodes were adjusted to produce approximately 400 responses per hour over a 24 hour period. When responding on all three levers had stabilized, animals were exposed to 8% oxygen for 24 hours and subsequently returned to 21% oxygen. Twenty four hours later, the ambient temperature was changed and the animals allowed to adapt to the new temperature for three days. Another exposure to 8% oxygen for 24 hours was followed by a return to 21% oxygen, terminating the experiment. The results indicate that exposure to hypoxia at high ambient temperature reduces self stimulation rates by 90% for the first 12 hours of exposure with a recovery to 50% of control during the next 12 hours. Exposure to hypoxia at low ambient temperatures increases self stimulation rates by 30% during the first 12 hours and by 20% during the next 12 hours. Food and water intake diminished in both conditions by 95%. This indicates that the behavioral effects of hypoxic stress are temperature dependent and that different underlying neurochemical alterations may be induced by the different exposure conditions.

1239 MURINE ADENOSINE 3',5'-MONOPHOSPHATE AND ETHANOL DEPENDENCE. William E. Askew and K. D. Charalampous. Dept. Psychiat., Baylor College of Medicine, Houston, TX, 77030.

Adenosine 3',5'-monophosphate (C-AMP) has been described as second messenger in peripheral as well as neural tissue. Adenylate cyclases are membrane bound and are thought to be involved with adrenergic receptor activity. Ethanol has a demonstrated affect on plasma membrane activity, thus presumably may affect receptor activity and adenylate cyclase and, hence, may affect C-AMP. We have studied the affect of ethanol dependence on C-AMP and related enzymes in mouse cerebellum and cerebral cortex using the pyrazole and non-pyrazole inhalation models. Cerebellar adenylate cyclase was not affected by ethanol dependence; however, cerebellar C-AMP levels were significantly depressed at 24, 48, and 72 hours. Cerebellar C-AMP phosphodiesterase activity was increased at 24, 48, and 72 hours. Adenylate cyclase activity in the cerebral cortex was increased at 48 and 72 hours with a resultant increase in C-AMP levels. Cortical C-AMP phosphodiesterase was not affected by ethanol dependence. Implications of these findings will be discussed.

1240 EFFECTS OF NICOTINIC AND MUSCARINIC COMPOUNDS ON BITING ATTACK IN THE CAT. G. G. Berntson, M. S. Beattie and J. M. Walker*. Lab. Comp. and Physiol. Psych., Ohio State Univ., Columbus, OH. 43212.

Predatory-like biting attack on a rat, as well as hissing, growling, and other threat behaviors, was induced in normally non-aggressive cats by systemic administration of the muscarinic agonist, arecoline (7-12 mg/kg). Arecoline-induced biting attack and threat were readily distinguishable on behavioral grounds, and were also differentiable in terms of the time-course of drug action, with biting attack generally appearing prior to the onset of threat. Nicotine was found to counteract the induction of aggressive behaviors by arecoline. Systemic administration of nicotine (0.5 mg/kg) prior to arecoline injection resulted in a significant reduction in elicited attack and threat behaviors, in terms of the latency to the onset and the total duration of the behaviors.

Nicotine was also found to be effective in suppressing natural predatory behaviors. Systemic nicotine injections (.075-.500 mg/kg) produced a dose-dependent suppression of biting attack in cats which demonstrated mouse-killing. This nicotine-produced suppression of attack did not appear to be due to the induction of general malaise, since attack suppression was seen in the absence of general behavioral inhibition, and doses of nicotine resulting in complete attack suppression had little effect on food intake. Further, pretreatment with hexamethonium (0.2 mg/kg), a peripheral nicotinic blocking agent, had little or no effect on nicotine-induced attack suppression, although it did effectively eliminate the transient peripheral effects sometimes observed with 0.5 mg/kg doses of nicotine.

These results are consistent with the view that muscarinic and nicotinic cholinergic systems may exert antagonistic control over some types of aggressive behaviors.

1241 EFFECTS OF INTRACEREBRAL ADMINISTRATION OF SALSOLINOL ON NOCICEPTIVE STIMULATION, NARCOTIC ANTAGONISM AND MORPHINE ANALGESIA IN MICE. K. Blum, E. Meyer*, J.E. Wallace*, H.A. Schwertner*, S. Futterman*, M. Hirst*, A. Marshall*, and M. Hamilton*. Departments of Pharmacology and Pathology The Univ. of Tex. Health Sci. Ctr., San Antonio, Tex., 78284, and Dept. of Pharmacology, Univ. of Western Ontario, London, Canada.

In spite of previous research there is increasing evidence for a relationship between opiate and alcohol dependence. A possible biochemical link between these two addictive substances may reside in the endogenous formation of alkaloids via condensation of acetaldehyde derived from ethanol and biogenic amines. Salsolinol, an endogenous alkaloid found in vivo following ethanol ingestion was tested for its effect on nociceptive stimulation as measured by the biting response in mice according to the tail clip method described by Haffner (1929). Salsolinol administered intracerebrally at 25, 50 and 100 µg/animal compared to a similar injection of cerebral spinal fluid (CSF) (at least $P < .05$) enhanced the number of bites elicited by tail clip pressure in mice. Morphine at 5 mg/kg i.p. reduced the biting response and this analgesic response by morphine was augmented by 50 µg/animal i.c. salsolinol. Naltrexone, a narcotic antagonist at 2.5 mg/kg blocked morphine analgesia. Unexpectedly, salsolinol interfered with this narcotic antagonistic action of naltrexone. Results are discussed with regard to possible common opiate receptor interactions and/or non-specific altered adrenergic effects by both salsolinol and morphine. (Supported by Air Force Grant #AFOSR-71-2074 and NIDA Grant #1-T01-DA00290-01.)

1242 THE EFFECTS OF METHAQUALONE ON THE SEIZURE SUSCEPTIBILITY OF MICE. W.O.BOGGAN, J.S.MEYER, R.M. STEINBERG* AND C. WORTHINGTON*. Dept. Psychiatry and Biochemistry, Med. Uni. of S.C., Charleston, S.C. 29401.

Methaqualone produces dose and time dependent decreases in seizure susceptibility to electricity, pentylenetetrazol, and sound. The effect on sound induced seizures was more prolonged than on the other agents. Methaqualone has previously been shown to disrupt temperature regulation and cause elevations in the concentration of plasma corticosterone. The anticonvulsant effects of methaqualone against electricity can be dissociated from its effects on temperature regulation and plasma corticosterone. Studies with SKF 525A, a drug known to block enzymes in the liver that metabolize drugs, demonstrate that methaqualone rather than a metabolite produced in the liver is responsible for its anticonvulsant effects. Tolerance to the anticonvulsant effects of methaqualone develops within one week after daily administration of the compound.

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1243 EFFECTS OF ACUTE AND CHRONIC LITHIUM TREATMENT ON HYPERACTIVITY IN RATS. D.R. Britton*, J.R. Bianchine* and S. Greenberg. Dept. of Pharmacology, The Ohio State University College of Medicine, Columbus, Ohio 43210

The "normothymic" action of lithium has been associated with a decrease in locomotor activity in manic patients. Equivalent effects on animal behavior have been largely confined to suppression of drug-induced hyperactivity. Male Long-Evans hooded rats were injected with either saline or lithium chloride (LiCl) and individually tested on automatic activity counters. Initial studies suggested that sub-toxic doses of LiCl had little effect on total daily activity of animals tested in their home cages. The animals were subsequently divided into two housing groups prior to testing: individually housed (IH) or housed in groups of three (GH). All rats were injected daily with either saline or LiCl (2 or 4 meq/Kg, i.p.) and tested for 30 min., 24 hr. after the last injection. In all cases GH animals were significantly more active than equivalently treated IH animals. 2 meq/Kg LiCl for 6 days produced a slight decrease in activity of IH rats compared to IH controls while there was significant suppression of the elevated activity of GH animals. Activity of 2 meq/Kg LiCl treated GH rats was equivalent to saline treated IH rats. Treatment with 2 meq/Kg LiCl for one day did not suppress activity. Preliminary data suggests that pargyline (75 mg/Kg, i.p., 5 hrs. prior to testing) suppresses activity in both GH and IH saline treated animals. Rats injected with 2 meq/Kg LiCl for 6 days did not show further decreased activity following pargyline and may be somewhat resistant to pargyline's suppressive effect. These data suggest a suitable model for studying the effects of lithium on locomotor activity and will be discussed in terms of possible involvement of catecholaminergic mechanism.

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1244 CNS MEDIATION OF THE AUGMENTATION OF LOCOMOTION AND STEREOTYPY DURING CHRONIC D-AMPHETAMINE ADMINISTRATION. Ronald G. Browne, Georve V. Rebec and David S. Segal. Dept. Psychiat., Sch. Med., UCSD, La Jolla, CA. 92093.

Previously we have demonstrated that repeated administration of d-amphetamine in rats produces a progressive augmentation of both locomotion and stereotypy. In order to determine if this augmentation results as a consequence of altered central mechanisms or because of changes in peripheral dispositional factors, we examined the behavioral effects of intraventricularly administered d-amphetamine, apomorphine, or norepinephrine in rats receiving long-term pretreatment with systemic d-amphetamine. Male rats were stereotaxically implanted with cannulae in the lateral ventricle and allowed to recover for at least one week before testing. The rats received four daily injections of either saline or d-amphetamine (2.5 mg/kg). Eight hours after the fourth injection, all animals were placed in the experimental apparatus. Twenty-four hours after the fourth injection, when augmentation can be demonstrated to systemically administered d-amphetamine, the animals were connected to the intraventricular infusion apparatus and allowed an additional hour of habituation. Various doses of the test drugs were infused into the ventricle at a rate of 20 µl/hr for up to four hours. The observed enhanced response to apomorphine implicates the involvement of dopaminergic receptor supersensitivity in the augmentation resulting from repeated d-amphetamine administration.

1245 THALAMIC AND BASAL FOREBRAIN SITES OF ACTION FOR AMPHETAMINE-INDUCED POTENTIATION OF THE REARING RESPONSE. Karen Smith Bryan and Gaylord D. Ellison. Dept. of Psychology, UCLA, Los Angeles, CA. 90024.

The rearing or standing-up response is a natural observing response emitted by small mammals. This behavior was electronically monitored in female hooded rats through the use of capacitance proximity circuitry, and the effects of systemic and central administration of d-amphetamine were studied. When administered intraperitoneally in low to moderate doses, d-amphetamine increased the number of rearing responses but shortened the average duration of each response. The effects of intracerebral microinjections of the drug were mapped using chronically implanted guide cannulae, and a number of sites were found to be inactive: ventral and lateral thalamus, hypothalamus, amygdala, hippocampus, lateral ventricles, and parts of the caudate-putamen complex. In contrast, injections placed unilaterally into thalamus in the region of the internal medullary lamina or into the region of the nucleus accumbens septi resulted in substantial increases in the amount of time spent rearing. The thalamic locus is of particular interest in that the pattern of response potentiation is distinctively different from that produced by systemic injections of d-amphetamine. This active area overlaps with the highly-labeled, wing-shaped structure described by Placidi et al (Brain Res., 38: 399, 1972) in their autoradiographic analysis of [^{14}C]amphetamine distribution.

1246 PHARMACOKINETICS OF CAFFEINE AND COFFEE: A COMPARATIVE STUDY. H. Dix Christensen. Dept. Pharm. Univ. of Okla. Health Sciences Center, Oklahoma City, Okla. 73190.

The pharmacokinetic parameters of caffeine as a drug, and as a dietary constituent (in coffee), were determined in ten male volunteers, 80 rats and 160 mice. Equivalent amounts of caffeine were given orally in paired sets of one, two, four, eight or sixteen (animals only) cups of coffee. The instant non-decaffeinated coffee contained 3.2% caffeine with 1.1 mg/kg caffeine being defined as one cup. Blood was collected before and at 5, 10, 30, 60, 90 min, and 2, 4, 6, 8, 12 and 24 hrs. post admin. Caffeine concentrations were measured by a specific caffeine radioimmunoassay. The kinetic parameters were found to be essentially dose independent.

In humans, the caffeine from coffee has a slightly longer elimination half-life of 4.1 hrs compared to 3.8 hrs for caffeine. With both treatment conditions, caffeine declined logarithmically over twelve hours. Absorption was rapid with an absorption half-life of 14 min and maximum plasma values, occurring at 53 min, were proportional to dose: C_{max}=1.63 (caffeine cups) -1.00 (R=0.91) and C_{max}=1.92 (coffee cups) -1.24 (R=0.87).

In rats, the caffeine from coffee elimination half life was 2.21 hrs compared to 2.09 hrs from caffeine. With both treatment groups, the decline was logarithmical over eight hrs. The absorption half life was 17 min and peak plasma values occurred at 62 min. The plasma values were proportional to dose C_{max}=1.44 (caffeine) -1.29 (R=0.97) and C_{max}=1.44 (coffee) - 0.66 (R=0.93).

In mice, the caffeine from coffee elimination half life was 1.37 hrs compared to 1.11 hrs from caffeine. The decline was logarithmically over 6 hrs. The absorption half life was 9 min, peak plasma values 36 min with regression values of C_{max}=1.08 (caffeine) - 0.72 (R=0.93) and C_{max}=0.90 (coffee)-0.49 (R=0.93). (Antiserum and caffeine -^{3}H were obtained from the Research Triangle Institute prepared under contract PH-43-NIGMS-65-657).

1247 A SIMPLE DEVICE FOR MEASURING IMPAIRED MOTOR FUNCTION IN MICE. Linda L. Coughenour*, Robert B. Parker* and John R. McLean. Research Laboratories Parke, Davis & Co., Ann Arbor, MI. 48106.

Measurement of the ability of mice to balance on a rotating rod or cone is often used as a measure of impaired motor function. In these procedures the mice must be trained prior to the test. In our laboratory the mice are given 3 training sessions on the day before and 1 session on the day of the test. Mice that fall from the 1 1/8 inch diameter rod, which rotates at 15 rpm, in 110 seconds or less are scored as having failed the test.

In the horizontal screen test described here, untrained mice weighing approximately 24 grams are placed on a battery of horizontal number 4 mesh screens, 13 X 13 cm. The screens are then rotated 180° so that the mice are on the bottom side of the screens. The majority of mice not treated with drugs, either climb to the top of the screens, or cling to the bottom during the 60 second period of the test. Two values are recorded: (1) the number of mice falling from the screens, and (2) the number of failures to reach the top, which is the sum of those remaining on the bottom of the screens and falling from the screens.

The ED_{50} values obtained for failure to reach the top of the horizontal screen were similar to those obtained with the rotorod, with the values for falling from the screen being slightly to considerably higher. At 60 minutes after oral administration of the test compounds, the ED_{50} values in mg/kg for the rotorod, failure to reach the top of the screen, and falling from the screen respectively were as follows: phenobarbital (66, 80, 85), chlordiazepoxide (46, 41, 57), thioridazine ($\approx$32, 38, 102), diazepam ($\approx$7, 6, 9). With both of the horizontal screen measures there were fewer control failures than in the rotorod procedure.

1248 EFFECTS OF CHLORDIAZEPOXIDE ON SPONTANEOUS RAPHE UNIT ACTIVITY IN THE ANESTHETIZED RAT. Mario Dalsass, Ronald Schoenfeld and Warren C. Stern. Squibb Inst. Med. Res., Princeton, N. J. 08540.

Chlordiazepoxide (CDP), an anxiolytic, markedly attenuates the effects of punishment on behavior in animal tests. Since serotonin has been proposed to play a key role in mediating the effects of punishment, the behavioral effects of CDP have been viewed as resulting from its disruption of central serotonergic functioning. We evaluated this view by examining the effects of i.v. and i.p. CDP on the activity of neurons in the raphe dorsalis (recorded extracellularly with glass microelectrodes) and control areas in chloral hydrate anesthetized rats. Results showed that CDP (i.v.) produced a dose-dependent decrease in raphe unit activity with the minimum effective i.v. dose being in the range of 0.1-0.8 mg/kg. Higher doses (0.9-1.7 mg/kg, i.v.) produced up to an 80% inhibition which lasted for at least 5-10 min. Complete suppression of firing was not seen at the maximum doses employed (1.7 mg/kg, i.v.; 20 mg/kg, i.p.). For the most part, control cells either adjacent to the raphe dorsalis, in the thalamus or neocortex failed to show changes in discharge rates following doses of CDP up to at least 3.5 mg/kg, i.v. The present data support prior suggestions that CDP produces a decrease in central serotonergic functioning. The possible involvement of brainstem glycine in mediating the effects of CDP on suppression of raphe unit activity and punished behavior will be discussed.

1249 CHOLINERGIC MOUSE-KILLING IN THE RAT. W. A. Dickinson* and R. A. Levitt (SPON: R. P. Lehr) Southern Illinois University, Carbondale, Illinois 62901

Mouse-killing by rats is a frequently employed model of predatory aggression. This type of aggression is characterized by a spontaneous and highly stereotypic attack elicited by natural prey stimuli such as mice. In a normal population of Long-Evans Strain rats, rats approximately 25% are natural or spontaneous mouse-killers. Smith, King, and Hoebel (Science, 1970, 167, 900-901) reported that they successfully converted non-mouse killer rats into mouse killers following bilateral injections of 50 micrograms of carbachol into the lateral hypothalamic area (LHA) of the rat brain. We have replicated and extended their work. As a result of a dose-response study we concluded that 20 micrograms of carbachol injected bilaterally in the LHA was the most effective dose for converting non-mouse killers into mouse killers. In addition, we observed that 94% of the carbachol-converted mouse killer rats also attack rat pups and adult rats while only 14% of the natural-mouse killer rats were observed to attack rat pups and adult rats in addition to mice. Finally, we also observed that 63% of the bites made by the natural-mouse killer group were localized in the cervical region of the mouse spine while this area only accounted for 23% of the bites made by the carbachol converted-mouse killer rats. This lack of stimulus specificity and non-stereospecific response topography suggest that carbachol induced aggression is more akin to an affective type of aggression than it is to predatory aggression.

1250 QUANTIFICATION OF ALCOHOL INTOXICATION AND WITHDRAWAL IN THE RAT. H.L. Edmonds, Jr. & S.I. Bellin*. Col. Pharmacy, Wash. St. U., Pullman, WA.99163

The magnitude of alcohol intoxication and withdrawal is usually quantified by a subjective rating of gross behavior. This imprecise technique makes assessment of drug action on these phenomena difficult. Therefore, objective changes in the level of cerebral excitability were used as indices of the severity of intoxication and withdrawal. The activity of the sensory system was measured by the electroshock startle threshold (EST) technique of Gibbins et al. (Psychopharmacol. 19:96, 1971). In addition, the cortical EEG was quantified by the use of a Grass summing integrator (Mardones et al., Res. Comm. Chem. Path. & Pharmacol. 10:273, 1975) which converted the voltage changes of the EEG into pulses. The number of these pulses per 5 min epoch, termed the voltage integrated index (VI^2), is proportional to the total energy content of the EEG.

Twenty adult male Sprague-Dawley rats were implanted with cortical screw electrodes. Physical dependence was produced in half of them by i.p. injections of ethanol (10% w/v) 3 times daily for 4 days. EST and VI^2 were measured every 4 hr after the last dose of ethanol for a 24 hr period. Data obtained from these animals were compared to a control group which received an oral sucrose solution isocaloric to the ethanol. The EST of the ethanol group was maximally increased by 33% at 8 hr post withdrawal (p.w.) when blood ethanol levels were very low and the animals demonstrated little or no behavioral signs of intoxication. By 24 hr p.w. the EST had decreased 38%. At this time half of the ethanol-treated rats exhibited marked tremors and all were hyperexcitable. A maximum increase in the VI^2 (39%) occurred 8 hr p.w. which paralleled the increase in EST. Surprisingly, during the hyperexcitable phase (16-24 hr p.w.), the VI^2 did not differ from control values. (Supported in part by a grant from Washington State, Initiative 171 funds.)

1251 CAFFEINE EFFECTS ON RETICULAR FORMATION NEURONS IN THE DECEREBRATE CAT. JESSE H. FORDE AND KENNETH R. HIRSH. N.Y. MEDICAL COLLEGE, DEPT. PHARMACOLOGY, VALHALLA, N.Y. 10595

IT HAS BEEN REPORTED PREVIOUSLY 1 THAT CAFFEINE (CAFF) SHORTENED THE INTERSPIKE INTERVAL (ISI) OF ACTIVITY RECORDED FROM SPONTANEOUSLY FIRING SINGLE NEURONS IN THE RETICULAR FORMATION (RF) OF THE BARBITURATE ANESTHETIZED RAT. WE NOW REPORT THAT CAFF (2.5 MG/KG) EXERTS A SIMILAR EFFECT IN THE MID-COLLICULAR DECEREBRATE, NON-ANESTHETIZED CAT. OF 45 NEURONS STUDIED 51% (23 CELLS) DISPLAYED A SIGNIFICANTLY SHORTENED ISI WHILE 24.4% (11 CELLS) HAD SIGNIFICANTLY PROLONGED ISI'S. THE REMAINING 24.4% (11 CELLS) WERE UNCHANGED IN THEIR FIRING RATE. THE DEGREE OF ISI REDUCTION AND THE TIME COURSE OF THE RESPONSE WAS SIMILAR TO THAT OBSERVED IN THE CASE OF THE RAT. OF THE 23 CELLS WHICH WERE SIGNIFICANTLY MORE ACTIVE AFTER CAFF THE AVERAGE ISI REDUCTIONS WERE TIME RELATED AS FOLLOWS: 23 MSEC AT 5 MIN., 31 MSEC AT 15 MIN., 23 MSEC AT 30 MIN. AND 17 MSEC AT 60 MIN. POST DRUG. CAFF AT 0.5, 1.0 AND 5.0 MG/KG DID NOT SIGNIFICANTLY ALTER ISI AT ANY TIME.

RECENTLY WE REPORTED 2 (IN SPONTANEOUS LOCOMOTOR ACTIVITY (SLA) STUDIES ON THE MOUSE) THAT CAFF REACHES A PEAK EFFECT AT RELATIVELY LOW DOSES (LESS THAN 20-25 MG/KG ORALLY) AND THAT FURTHER INCREASES IN DOSE EVOKE SMALLER CHANGES IN SLA. IT APPEARS THAT CAFF EXHIBITS A SELF LIMITING CHARACTERISTIC WHICH CARRIES OVER INTO SINGLE RF NEURONS AS WELL, EITHER IN THE PRESENCE OR ABSENCE OF ANESTHESIA.

1-NEUROSCIENCE ABST. 1974: HIRSH, K., FORDE, J. & PINZONE, M.
2-FED. PROC. 33, 466, 1974: HIRSH, K., PINZONE, M. & FORDE, J.

1252 THE BEHAVIORAL EFFECTS AND TISSUE STORAGE PROFILE OF OF PHENCYCLIDINE IN THE RAT. Carole D. Hansult and Stuart James.* Dept. Psych., SUNY at Binghamton, Binghamton, N.Y. 19301

Fifty-four adult female rats were each injected (I.P.) with 75 mg/kg phencyclidine hydrochloride. Just prior to injection animals were tested for righting reflex, corneal sensitivity and reaction to tail pinch. Groups of six subjects were sacrificed at 1,2,3,4,8,16,24,48 or 72 hours after drug. Each animal was retested just before sacrifice for each of the behavioral tasks. Samples of blood, liver, kidneys, fat pads, muscle and brain were collected and then assayed for phencyclidine via gas chromatography. Results show rapid uptake of the drug by all tissues with storage of up to three days by the brain and fat pads. Tissue levels in liver, kidney and muscle fall quickly but rise again days later as the brain and fat begin to release the drug. Behaviorally there are no significant effects on corneal sensitivity but both righting reflex and tail pinch reaction are lost for most of the test period. Extensive hemhorraging of the liver, brain, kidneys, eyes and nasal passages was noted in all subjects by the beginning of the sixteenth hour and was still evident in many cases at the final test period.

1253 DIETARY CADMIUM: EMBRYOTOXICITY AND BEHAVIORAL EFFECTS IN RATS. L. Hastings*, H. Choudhury*, G.P. Cooper, and H. Petering*. Dept. Environ. Health, U. Cincinnati Col. Med., Cincinnati, OH. 45267.

Male and female weanling rats were given distilled water containing 17.2 μg/ml of cadmium chloride (Cd^{++}) to drink for 90 days. They were then mated and Cd^{++} exposure continued throughout the period of gestation. Control rats were treated identically except that they received distilled water only. Beginning at parturition and continuing for the 21 day lactation period all rats were given deionized water. The pups were weaned onto Purina Laboratory Chow and tap water and maintained on this diet for the remainder of the experiment. Although the Cd^{++}-exposed pups were significantly smaller at birth than coetaneous controls, their body weight gain during lactation was comparable to the control pups. Whole pup body metal analysis showed that control pups had approximately 40% more iron in their body than the Cd^{++}-exposed pups. Spontaneous Locomotor Activity (SLA), as measured by wheel running was significantly depressed during a five week period beginning at 35 days of age. Food and water consumption and body weight were essentially equivalent in both groups throughout the period. Beginning at approximately 150 days of age the two groups were tested on the acquisition of a position habit task. The task was run in an operant chamber and involved pressing the left bar in a two-bar choice situation. The two groups showed no difference in either acqusition or reversal of the original task. Thus the offspring of rats exposed to chronic low-level Cd^{++} show depression of SLA but no impairment in the acqusition of a simple position habit. The absense of any impairment in acquiring the position habit may reflect more the relative ease with which rats learn this task rather than a lack of central neurotoxic effects. (Supported by NIEHS grant ES-000159).

1254 CORTICAL SENSORY EVOKED POTENTIALS AND THE STARTLE REFLEX IN RHESUS MONKEYS AND CATS RECEIVING LITHIUM. George R. Heninger and Michael Davis, Dept. Psychiat. Sch. Med., Yale Univ., New Haven, CT 06508.

Increased amplitude of early components of the scalp recorded somatosensory evoked response and slowing of sensory-motor speed occur concomitantly prior to and during symptom improvement in patients on lithium (Heninger, G.R., J. Psychiat. Res. 10:156,1974). To investigate the neural basis of these findings, sensory evoked potentials and the amplitude of the acoustic startle reflex were measured in rhesus monkeys and cats given lithium, using doses and serum lithium levels within the range used clinically. Startle amplitudes were recorded with special cages in which an accelerometer sensed cage movement during a specified time after the stimulus. Several different intensities of white noise bursts were used to elicit startle. Average evoked cortical responses to percutaneous median nerve stimulation and to clicks were recorded from dural, intracortical and subcortical electrodes. Startle amplitude increased in both species following lithium. Increased startle did not directly relate to maximum serum lithium levels, but occurred 3-5 days later. Amplitudes of dural and intracortical evoked responses to auditory and somatosensory stimuli also increased in both species following lithium. Evoked response changes were manifest in earlier portions of the wave form. The time course of startle reflex and evoked response changes were highly correlated, approaching a day by day correspondence between the two measures. Sodium chloride administration produced no changes in either startle or evoked potentials. Previous studies suggest that lithium effects on sensory evoked potentials originate in the cortex. The temporal concurrence of evoked potential and startle changes following lithium suggest that they may both result from a reduction in sensory motor inhibitory cortical function, a mechanism which could also be operant in the slowing of sensory motor speed of patients.

1255 CNS STIMULATION EVOKED BY CAFFEINE CAN BE PREVENTED BY PRIOR ADMINISTRATION OF NICOTINIC ACID. KENNETH HIRSH, MARILYN PINZONE AND JESSE FORDE. (SPON: DOROTHY CHOU) N.Y. MEDICAL COLLEGE, DEPT. PHARMACOLOGY, VALHALLA, N.Y. 10595.

CAFFEINE (CAFF) IS KNOWN TO SHORTEN PENTOBARBITAL SLEEPING TIME IN MICE. ANIMALS RECEIVING NICOTINIC ACID (NA) CONCURRENTLY WITH CAFF (P.O.) DISPLAYED A REDUCED OR COMPLETELY ELIMINATED CAFF EFFECT. TO DETERMINE THE LEVEL OF THIS INTERACTION WE STUDIED IT IN RATS UNDER PENTOBARBITAL, AND IN THE MIDCOLLICULAR DECEREBRATE CAT PREPARED UNDER HALOTHANE ANESTHESIA. IN BOTH OF THESE PREPARATIONS ADMINISTRATION OF NA PRIOR TO CAFF PREVENTED THE INCREASED ACTIVITY OF SINGLE RETICULAR FORMATION (RF) NEURONS USUALLY RECORDED WHEN CAFF IS GIVEN ALONE. NA GIVEN AFTER CAFF WAS UNABLE TO ALTER THE ON-GOING CAFF RESPONSE. NA ADMINISTERED ALONE DID NOT CAUSE ANY OBSERVABLE CHANGES IN EITHER SLEEP TIME OR FIRING RATE OF RF NEURONS. IT IS CONCEIVABLE THAT NA IS OPERATING AS A CAFF ANTAGONIST AT THE CELLULAR LEVEL BY ALTERING CAFF EFFECTS ON CYCLIC AMP LEVELS. ONE MIGHT SPECULATE ALONG THESE LINES THAT A DECREASE IN CYCLIC AMP SYNTHESIS BROUGHT ABOUT BY NA COULD PREVENT AN ACCUMULATION OF CYCLIC AMP BROUGHT ABOUT BY LATER ADMINISTRATION OF CAFF WHICH IS A WELL KNOWN PHOSPHODIESTERASE INHIBITOR. THIS IS CONSISTENT WITH THE REQUIREMENT OBSERVED IN OUR STUDIES THAT NA BE GIVEN EITHER SIMULTANEOUSLY WITH OR PRIOR TO CAFF IN ORDER TO BE EFFECTIVE IN PREVENTING THE CAFF EVOKED RESPONSES.

1256 AN ANIMAL BEHAVIOR MODEL FOR STUDYING THE ACTIONS OF LSD AND RELATED HALLUCINOGENS. Barry L. Jacobs, Michael E. Trulson, and Warren C. Stern, Dept. Psychol., Princeton Univ., and Squibb Inst. Med. Res., Princeton, NJ 08540.

In the course of examining the dose-response relationship for the behavioral effects of LSD in the cat, we discovered that, in addition to large increases in investigatory and hallucinatory responses, two behaviors, not previously reported, are emitted with a high probability under LSD. For this reason we hypothesized that these behaviors could serve as an animal model for the actions of LSD, and related drugs, in man. Beginning from a baseline of essentially zero in saline-treated animals, limb flicks and abortive grooming increase in frequency in direct relation to the dose of LSD administered (10, 25, 50, and 100ug/kg, i.p.). Limb flicks are a species-specific behavior seen in normal cats almost exclusively in response to placing a foreign substance, such as water, on the hindpaw or forepaw. In abortive grooming, the cat orients to the body surface as if to groom but does not emit the consummatory grooming response (bite, lick, or scratch), or emits the response in midair. The specificity of these behavioral changes is indicated by the fact that they are never seen in response to other classes of psychoactive drugs and control compounds such as d-amphetamine, atropine, THC and brom-LSD. They are, however, produced by compounds which are structurally and functionally related to LSD, such as methysergide and psilocybin. Paralleling important parameters of the action of LSD, these behavioral changes are long-lasting, and show tolerance that is both rapidly developing and of long duration.

1257 SOME BEHAVIORAL AND BIOCHEMICAL PROPERTIES OF ENKEPHALIN AND α-ENDORPHIN. Yasuko F. Jacquet, Neville Marks, and Frederick Stern*. Res. Inst. Neurochem., Rockland Res. Inst., Ward's Island, NY . 10035.

Previous work from this laboratory has established two CNS sites as yielding reliable behavioral effects following morphine microinjection. These are1) the periaqueductal gray, where a morphine microinjection resulted in pronounced analgesia, accompanied by an explosive hyper-reactivity to previously neutral auditory and visual stimuli (Science 185: 1055, 1974) and 2) the midbrain reticular formation,where a morphine microinjection resulted in bursts of rapid ipsilateral rotation in response to previously neutral auditory and visual stimuli (Science 192: 261, 1976).

Enkephalins and α-endorphin (cf. Guillemin, et al, C.R. Acad. Sci. Paris Ser.D., 282: 783, 1976), peptides with morphine-like actions in vitro (guinea pig ileum, binding to brain extracts) were microinjected directly into these 2 CNS sites of chronically cannulated rats in doses ranging from 4 - 80 ug to see if they mimicked the action of morphine. No analgesia or other morphine-like central effects were detected throughout a 2 h observation period. The only noticeable CNS effects were the appearance of sedation and loss of righting reflex following restriction of visual input, which occurred within 10 min and lasted approximately 2 h.

Breakdown of Met-enkephalin and α-endorphin (sequences 61-65 and 61-76 of β -LPH) was measured in vitro following incubation with rat brain extracts and sera. Enkephalins (Met and Leu) were rapidly degraded within 1 - 10 min, with appearance of N-terminal Tyr and diglycine; α-endorphin and Met-enkephalin amide were more slowly degraded. Data on breakdown may account for the absence of morphine-like central effects, but does not exclude the possibility that the formation of intermediates can result in other central effects such as noted above.

1258 MORPHINE, KETAMINE AND HARMALINE INFLUENCES ON RAT CEREBELLAR 3', 5'-CYCLIC GUANOSINE MONOPHOSPHATE LEVELS. J.B. Katz* and G.N. Catravas. Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014.

Harmaline - a known elevator of cerebellar 3', 5'-cyclic guanosine monophosphate (cGMP) levels - produced a 500 percent rise in cGMP, from 5 to 25 pmoles/mg protein when injected at 30 mg/kg, i.p..This rise was partly blocked by morphine pretreatment, but even 45 mg/kg morphine failed to block harmaline-induced tremor and to reduce the cGMP level below twice the control value. Morphine injected 10 min after harmaline reduced cGMP more potently, but some tremor and cGMP rise persisted with 45 mg/kg morphine. These harmaline-morphine relations were identical in rats chronically treated with morphine, although such rats lacked the analgesic response to morphine. Ketamine strongly reduced harmaline-elevated cGMP levels to 30 percent of the control value and blocked or abolished harmaline tremor when injected 20 mg/kg, i.p., before or after harmaline. Isoniazid (INH)(300 mg/kg, i.p.) elevated cerebellar cGMP to 700 percent of control levels. Phosphodiesterase activities in soluble fractions of cerebellar homogenates were equal in control rats and in morphine, harmaline, and INH-treated rats. Addition of 10^{-4} M glutamate or gamma-aminobutyric acid (GABA), both putative cerebellar transmitters, did not alter phosphodiesterase activity. Particulate cerebellar guanylate cyclase activities were equal in control and morphine-treated rats; however, guanylate cyclase activity was increased approximately 30 percent in rats receiving harmaline or INH.

1259 THE EFFECT OF ETHANOL ON THE CEREBRAL OXIDATIVE METABOLIC RESPONSE TO DIRECT ELECTRICAL STIMULATION. Joseph C. LaManna, B. Wyatt Younts jr* and Myron Rosenthal. Department of Physiology and Pharmacology, Duke University Medical School, Durham, N.C. 27710.

The effect of ethanol on oxidative metabolism of intact cerebral cortex was monitored in cats and rats (cerveau isolé preparations) by NADH fluorometry and dual wavelength reflection spectrophotometry. Low ethanol doses (below 1g/kg) produced an increased level of reduced NAD and cytochrome a,a_3. This effect was common to other CNS depressants (e.g. phenobarbital, chlorpromazine) and appears due to decreased tissue activity. Higher ethanol doses (1-2g/kg) produced an increased level of reduced cytochrome a,a_3, but an increase in oxidized NAD. Acetaldehyde at all effective doses produced similar alterations as high ethanol doses. For this reason, the actions of ethanol and acetaldehyde on the metabolic responses to direct cortical stimulation were compared. Previous to administration of these drugs, short trains of pulses (2 dec duration with pulse width at 0.5 msec, 20 Hz at approximately 5-10 volts) were accompanied by transient increases in the level of oxidized NAD which became re-reduced and returned to baseline within 20 seconds. Low ethanol and acetaldehyde doses slowed the rate of NAD oxidation, an effect similar to that produced by ouabain microinjected under the cortical surface. Doses of ethanol or acetaldehyde near LD_{50} slowed both the rate of oxidation and the rate of re-reduction of NAD following stimulation. These data indicate that some neurochemical actions of ethanol are due to acetaldehyde and that an inhibition of Na^+, K^+-ATPase may be involved in the ethanol effect on electrophysiological activity. (These studies were supported by PHS grant NS 10384 and a grant from the North Carolina Alcoholism Research Authority).

1260 IN VITRO STUDIES OF LITHIUM IN HUMANS. Kenneth W. Lieberman* and Peter E. Stokes. Department of Psychiatry, Cornell University Medical Center, New York, New York 10021.

The usefulness of lithium (Li) in the treatment of the manic phase of the manic-depressive illness is generally accepted, but the possibility that Li may have some potential as an indicator of some underlying biological basis for the development of affective disorders has not been widely explored. An in vitro procedure has been developed to determine the quantity of Li ion which enters into the erythrocyte as a function of time. Preliminary studies have been conducted using the newly developed technique to obtain some basic data relevant to the uptake of the Li ion into erythrocytes derived from psychiatric normals and manic-depressives. A linear relationship existed between the quantity of Li ion which entered the erythrocyte and time from hours 1-4 of the Li uptake procedure for the psychiatric normals and manic-depressives; the relationship was non-linear during the first hour for both groups. Less Li appeared to be taken up in the manic-depressive erythrocyte than in the psychiatric normal erythrocyte as a function of time. When the two experimental groups were subdivided into male and female, no difference in Li ion uptake was noted between the psychiatric normal and manic-depressive males. A smaller quantity of Li was, however, present in erythrocytes obtained from manic-depressive females than psychiatric normal females. Additional work must be done in order to further substantiate there findings, but the implications are clear that some factor(s) responsible for governing ion transport across a membrane may be related to the etiology or development of affective disorders.

1261 AMPHETAMINE MODIFICATION OF LIMBIC LESION INDUCED BEHAVIOR. Irwin Lourie* Michael M. Krieger* (SPON: N. S. Thampi). Research Dept., Norristown State Hospital, Norristown, PA. 19401

Bilateral radiofrequency lesions were placed in dorsal Hippocampus(H), Septum(S), and Amygdala(A) in groups of male 420-460 gm NSH rats [n=10 for each group and controls(C)]. Following a 14-day recovery period animals were run daily in an open field paradigm consisting of 36 min of individual exploratory activity (Exp) and 36 min of paired social interaction (SI). When the behavior had become relatively stable (5 days) behavioral data was collected for 3 baseline days followed by a drug day with 2 mg/k dl-amphetamine sulfate (Amp) i.p. 30 min prior to assay. A time sampling method was used to collect 3 one-min samples of Exp and 3 two-min samples of SI. Data presented are: for Exp - total activity, ratios of motor (M) to non-motor (NM) components and rates of habituation; for SI - total activity and ratios of interactive (I) to non-interactive (NI) behavior. All lesions showed deficits in total Exp: H, S and A being 89, 75 and 59% of controls, M/NM being 0.4 for all treatments. Habituation rates were increased for all lesioned groups 162, 172 and 324% for H, S and A. The effect of Amp was to normalize all behavior to the level of C with one exception, a hyperreactive response of 130% in a motor score for S. M/NM increased for all groups to 0.8. Total activity in I shows deficits in H, S and A of 88, 64 and 77% with corresponding deficits in SI of 71, 54 and 35%(C).I/NI for C, H, S and A are 1.0, 0.7, 0.8 and 0.3. The effect of Amp is to normalize total activity to that of C. I/NI, however, remains at pre-drug level. Thus Amp modifies motor activity associated with limbic system influence but not the probability of occurrence of species specific social behavior.

1262 INHIBITION OF DIMETHYLTRYPTAMINE BIOSYNTHESIS BY DBN. Lewis R. Mandel, Merck Institute for Therapeutic Research, Rahway, N.J. 07065

1,5-Diazabicyclo [4.3.0] non-5-ene (DBN) has been found to be a potent inhibitor of the dimethyltryptamine (DMT) forming enzyme indoleamine-N-methyltransferase (INMT). In vitro the compound produces 50% inhibition of the rabbit lung enzyme at 2 μM; it is non-competitive with respect to N-methyltryptamine (NMT). DBN also inhibits the S-adenosylmethionine dependent conversion of 5-methoxy-NMT and N-methylserotonin to 5-methoxy DMT and bufotenin 80% at 0.2 mM. While INMT activity of monkey and human lung, rabbit brain and liver is also inhibited by DBN, it does not block adrenal phenethanolamine- or lung imidazole-N-methyltransferases at 0.2 mM. When administered to rabbits at 3-30 mg/kg i.v. or 60-300 mg/kg orally DBN reduces the specific activity of the lung INMT up to 85%. Inhibition decreases in a time-dependent fashion measured from 10 min to 4 hr after dosage. When enzyme preparations from DBN treated rabbits were dialyzed, the specific activity returned to control values (ca. 80,000 cpm DMT/mg protein/hr) suggesting a reversible type of inhibition. The decrease in INMT activity was associated with up to a 95% inhibition in the in vivo biosynthesis of C^{14}-labeled DMT in rabbit lung and brain following the i.v. injection of C^{14} NMT. The level of carotid arterial DMT (20-40 ngm/ml) appearing over 1 minute after i.v. injection of NMT (7-12 mg/kg) was also reduced 90%. DBN had no activity in a variety of other in vitro or in vivo assays. As DMT has been implicated in the etiology of schizophrenia, a compound with the biological profile of DBN would be of interest in testing the indoleamine-transmethylation hypothesis.

1263 HYPERTHERMIA INDUCED IN THE FREE-MOVING RAT BY THE INTRACEREBRAL MICRO-INJECTION OF MORPHINE SULPHATE. Gregory E. Martin, Dept. Neurophysiology, Walter Reed Army Institute of Research, Washington, D.C. 20012.

In previous experiments, a hypothermic response was evoked by the cerebral microinjection of 50 µg of morphine sulphate (MS) into the hypothalamic/preoptic region (APO) of the restrained rat (Lotti, Lomax, & George, J. Pharm. Exp. Therap. 1965, 150:135). However, the direction of the thermal response following the IP injection of MS, is a function of the degree of constraint imposed on the rat. After an IP injection of MS, hypothermia results in the restrained animal whereas hyperthermia results in the free-moving rat. Hence, the effect of MS microinjected in the APO of the free-moving rat was examined. In seven rats, two 24 ga stainless steel cannulae were implanted in the APO. All microinjections were made in a volume of 0.5 µl via a 30 ga needle. MS, dissolved in an artificial cerebrospinal fluid (CSF), was injected in doses of 1.0 (n=8), 10.0 (n=12), 20.0 (n=3), and 50.0 µg (n=3). As a control the CSF was injected alone. In addition, microinjections of the 20.0 µg dose were made in the rat restrained in a plastic holder. In the free-moving rat, a pronounced hyperthermia was observed following the injection of MS at 14 sites in the APO. The mean peak rise (MPR) in temperature following the 1.0 µg injection was 2.5°C. Similarly, increases in core temperature were observed after the 10.0 (MPR=3.0°C), 20.0 (MPR=2.7°C), and 50.0 µg (MPR=3.0°C) dose levels. The MS-induced hyperthermia was interrupted by the intracerebral injection of naloxone. In the present experiment, however, only a slight hyperthermia (MPR=1.1) was observed in the rat restrained in the plastic holder when 20 µg MS was injected in the APO. These data indicate that a hyperthermic response is elicited by the microinjection of MS into the APO of the free-moving rat which can be attenuated by restraining the animal.

1264 LACK OF DEVELOPMENT OF SUPERSENSITIVITY OF PITUITARY DOPAMINE RECEPTORS FOLLOWING CHLORPROMAZINE. Meltzer, H.Y., Goode, D.J., Paul, S.*, Fang, V.S.* and Kadjan, B.*. Dept. Psychiatry, Univ. Chgo. Pritzker Schl. Med. and the Ill. State Psychiatric Institute, Chicago, Illinois.

There have been numerous biochemical, electrophysiologic and behavioral demonstrations of apparent supersensitivity of dopaminergic receptors of the neostriatum following chronic neuroleptic administration in rats. We investigated the possibility of development of supersensitivity in the hypothalamo-pituitary axis following chronic administration of Chlorpromazine (CPZ) in man and rats by quantifying the decrease in serum prolactin (PRL) levels produced by apomorphine (APO), a dopamine agonist. Since PRL secretion from the pituitary is inhibited by dopamine, APO decreases serum PRL. Five schizophrenics who had not been previously or recently treated with neuroleptics were given APO 0.75 mg subcutaneously. PRL levels were determined in a series of samples from 30 min prior to APO to 2 hrs after APO. Another subject, who had early tardive dyskinesia, a condition which has been associated with supersensitivity of the dopamine receptors of the neostriatum, was also studied in this manner. CPZ was administered for 14 days and in doses up to 400 mg/day, stopped for 5 days and the APO readminstered. There was no significant difference in the fall in serum PRL levels before and after CPZ.

Groups of 5 male Sprague-Dawley rats weighing 150-175 gms were given saline or CPZ 10 mg/kg i.p. b.i.d. for 10 days. Five days after stopping saline or CPZ, they were given APO 1.0, 2.5 or 5.0 mg/kg i.p. and the serum PRL levels were determined 30 min later. There was no significant difference in serum PRL levels in the saline or CPZ-treated rats. It thus appears that the dopamine receptors in the pituitary or hypothalamus which regulate PRL secretion do not develop supersensitivity after neuroleptic administration despite the fact that many similarities in drug response have been demonstrated for the pituitary and neostriatal dopamine receptors.

1265 PHARMACOLOGICAL DIFFERENTIATION BETWEEN ATTACK AND DEFENSIVE-SUBMISSIVE REACTIONS IN MICE, RATS AND SQUIRREL MONKEYS: EFFECTS OF Δ^9-TETRAHYDRO-CANNABINOL, ALCOHOL AND PSYCHOMOTOR STIMULANTS. Klaus A. Miczek. Dept. Psychol., Carnegie-Mellon Univ., Pittsburgh, PA. 15213.

Species-specific patterns of attack, threat, defense, submission and flight were generated by introducing a strange male conspecific animal into the home cage of single or grouped mice, rats, or squirrel monkeys. Δ^9-Tetrahydrocannabinol (THC), alcohol, d-amphetamine, cocaine, or L-DOPA were administered either to the intruding animal or to the resident male. Latency, duration and frequency of 10-20 discrete acts and postures characteristic of attack and threat by the resident animal, and of defense, submission or flight by the intruding animal, were recorded independently for each combatant during 5-10 min. of intense fighting episodes. (1) Low doses of amphetamine (0.1, 0.25 mg/kg), L-DOPA (5, 10 mg/kg), alcohol (0.5 g/kg) enhanced attack behavior in rats, but not in mice, whereas higher doses suppressed it. THC and cocaine consistently suppressed attack in a dose-dependent manner in mice, rats and squirrel monkeys, THC being very effective at low doses (0.25 - 2.0 mg/kg). (2) The same drugs affected defensive and submissive reactions at dose levels that were 5-10 times higher than those altering attack. Amphetamine (>1.0 mg/kg), cocaine (>4.0 mg/kg), L-DOPA (>100 mg/kg), alcohol (1.5 g/kg), and THC (4.0 mg/kg), when given to the intruding animal, led to increased and often more injurious attacks by the non-drugged resident animal.

Attack and threat behavior is considerably more sensitive to drug action than defensive-submissive reactions. Fighting behavior by rats and squirrel monkeys is altered at doses 3-8 times smaller than that in mice, suggesting a possible differentiation between territorial and dominance fighting. (Supported by USPHS DA-00722).

1266 INCREASED SPECIFIC NEUROLEPTIC BINDING AFTER CHRONIC HALOPERIDOL IN RATS. P. Muller and P. Seeman, Pharmacology Department, University of Toronto, Toronto, Canada.

It is known that after chronic haloperidol administration rats exhibit more stereotyped chewing in response to apomorphine (Tarsy and Baldessarini, Neuropharmacol., 13, 927, 1974).

In order to test whether chronic haloperidol might elicit this changed sensitivity by altering the number of neuroleptic/dopamine receptors in the striatum, we measured the specific binding of ^{3}H-haloperidol in rat striatum after 6 weeks of 1.5 mg/kg haloperidol(p.o.), using a modification of previous methods (Seeman et al., Proc. Nat. Acad. Sci., 72, p. 4376, 1975). The final mixtures were filtered through GF/C filters and washed. Stereospecific binding was defined as that amount of ^{3}H-haloperidol bound to the striatal crude homogenate in the presence of 100 nM (-)-butaclamol minus that bound in the presence of 100 nM (+)-butaclamol.

Following the chronic haloperidol treatment there was an increase of specific binding of ^{3}H-haloperidol. Preliminary experiments indicate an increase of 30 to 50%.

If the neuroleptic and dopamine receptors are one and the same, the behavioural supersensitivity to apomorphine (following chronic haloperidol) may be explained by the increased number of neuroleptic/dopamine receptors.

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1267 LITHIUM TRANSPORT BY RAT BRAIN SYNAPTOSOMES. David G. Ostrow*, G. N. Pandey, John M. Davis and D. C. Tosteson* (SPON: Klaus Unna). Ill. State Psychiat. Institute, Chicago, Ill. 60612 and Univ. Chicago, Chicago, Ill. 60637.

We have studied lithium uptake by rat brain synaptosomes in order to determine whether or not the characteristics of lithium transport by synaptosomal membranes are similar to those of lithium transport by red cells (Pandey, et. al., these proceedings). At 37°C in Na^+ medium containing 20 mM LiCl, lithium entry into synaptosomes was rapid, reaching equilibrium levels within 5 to 10 minutes. As in red cells, lithium appears to be transported by three major mechanisms: a (Na^+-K^+)-transport ATPase mediated pathway, a Na^+-gradient dependent Na^+-Li^+ exchange pathway, and a leakage pathway. The (Na^+-K^+)-ATPase dependent pathway is ouabain sensitive, requires low levels of K^+ (2-3 meq/l) and glucose for optimal activity, and is completely inhibited by raising the external potassium concentration to 10 meq/l. The Na^+-Li^+ exchange system was completely inhibited by phloretin (10^{-4}M), resulting in a higher equilibrium concentration of Li^+ in its presence. This phloretin effect was present in Na^+ but not K^+ medium. These results suggest that in rat brain synaptosomes, as in human red cells, the major proportion of lithium entry is via the ouabain sensitive (Na^+-K^+)-transport ATPase system and leakage pathways, while efflux occurs mainly via the phloretin sensitive Na^+-Li^+ exchange system.

1268 THE DEVELOPMENT OF TOLERANCE TO TOLUENE IN AN ANIMAL MODEL FOR HUMAN SOLVENT ABUSE. R. G. Peterson, Dept. of Neurobiol. and Anatomy, J. V. Bruckner,* Dept. of Pharm.The Univ. of Texas Med. Sch. at Houston, Houston, TX 77025.

Mice (Sprague-Dawley ICR) were exposed 5 times per week for 3 hours for 8 weeks to 15 mg of toluene per liter of air (4000 PPM). A series of control (no prior toluene exposure) and experimental-animals (5 of each) were tested during exposure at 1, 2, 4, and 8 weeks and at two weeks post exposure with selected performance tests, to reveal development of tolerance to depressant effects of toluene (Irwin, Psychopharmacologia, 13:222, 1968). The tests used included visual placing, grip strength, pinna reflex, corneal reflex, wire maneuver, tail pinch and righting reflex. Each of these tests was scored on a scale of 0-8 with 0 representing inability to perform and 8 representing the most proficient performance. The animals were tested after 0, 15, 30, 45, 60, 120, and 150 minutes of exposure. The scores obtained were evaluated by analyzing each test individually and by averaging all the scores of each animal at each time to obtain an overall performance score. Analysis of variance was performed on data of all groups of animals to reveal statistical significance. For the overall scores, significant difference between control and experimental groups was demonstrated after 2 and 8 weeks of exposure, and 2 weeks post exposure. Analysis of individual tests scores showed little difference between controls and experimental animals' performance at 2 and 4 weeks of exposure except in the wire maneuver test, where there was a highly significant difference at 4 weeks. After 8 weeks of exposure, and 2 weeks post exposure, there was a significant difference in almost all individual test scores, with experimentals scoring higher than controls. These experiments show that tolerance to toluene can be demonstrated in an animal model of human solvent abuse. (Supported by NIH Contract #271-75-3067)

1269 DEMONSTRATION THAT IMIPRAMINE POTENTIATES THE EXCITATORY EFFECT OF INTRAVENTRICULARLY-ADMINISTERED NOREPINEPHRINE ON INTRACRANIAL SELF-STIMULATION. B. P. H. Poschel* and F. W. Ninteman* (SPON: D. A. McCarthy). Parke, Davis & Co., Ann Arbor, MI 48106.

It has been known for some time that intraventricular injections of 10 µg of l-norepinephrine (NE) will moderately increase rates of medial forebrain bundle self-stimulation in rats. Theoretically, pretreating the rats orally with imipramine (10-20 mg/kg, 90 min earlier) should potentiate this effect of NE much in the same manner that imipramine potentiates the excitatory effect of orally administered amphetamine on self-stimulation. However, in actual self-stimulation tests of imipramine and intraventricular NE this expected action never occurs; instead, the normal excitatory action of NE is blocked. However, we have discovered that the expected potentiating action of imipramine in this experiment can be made to occur by orally administering 10 mg/kg of ripazepam (CI-683**) shortly after the intraventricular injection of NE. These results suggest that imipramine sensitizes both behaviorally excitatory and behaviorally inhibitory neurons. The anti-anxiety drug ripazepam suppresses the inhibitory system (probably serotonergic and/or gabaergic) leaving imipramine's sensitizing action on the excitatory system (probably noradrenergic) unopposed. Thus, under these conditions the expected potentiating action of imipramine on the excitatory effect of intraventricular NE is free to occur.

**B. P. H. Poschel, D. A. McCarthy, G. Chen, and C. R. Ensor. Pyrazapon (CI-683): a new antianxiety agent. Psychopharmacologia (Berl.) 35, 257-271 (1974).

1270 COMPARISONS OF BEHAVIORAL CHANGES IN SELECTED MEMBERS OF PRIMATE SOCIAL COLONIES INDUCED BY CHRONIC AMPHETAMINE & PHENCYCLIDINE (PCP)TREATMENT. K L Preston*, R F Schlemmer, Jr, D L Garver, J A Jackson*, J P Bederka, Jr*, & J M Davis (SPON: N L Katz). Ill State Psychiatric Inst & U of Ill Med Center, Chicago, IL 60612.

Although each has diverse pharmacologic actions, amphetamine & PCP both have been implicated in the induction of schizophreniform psychoses. To compare these agents, selected members from stable, adult primate social colonies of 4-6 monkeys (Macaca arctoides) received chronic administration for 2 wks of either d-amphetamine (d-A), 1.6 mg/kg, in time-release form nasogastrically every 12 hours, or PCP, 25 µg/kg, i.m. 15 mins prior to observation. A "blind" observer quantified & recorded colony behavior for a 1 hr observation period at designated times. A behavioral baseline was recorded similarly prior to each expt. Both d-A & PCP induced stereotyped behavior (SB) in all treated animals. Most of the SB was non-social (solitary) in nature in the majority of monkeys treated with either agent. However, qualitative differences in the SB induced by each agent were noted. Administration of either d-A or PCP resulted in a decrease in social grooming in most treated monkeys, particularly toward the end of chronic treatment. Huddling & resting with eyes open decreased with d-A treatment, but remained unchanged or increased with PCP treatment. Prior & concomitant treatment with pimozide (Pmz), an agent that preferentially antagonizes dopamine (DA) receptors, prevented the development of either d-A or PCP-induced SB. The results of this study demonstrate that some behavioral similarities do exist between these 2 psychotomimetic agents. Since Pmz antagonized both d-A & PCP-induced SB, it appears that d-A & PCP may both possess central DA stimulant properties.

1271 AMPHETAMINE AND METHYLPHENIDATE: NEUROCHEMICAL, ELECTROPHYSIOLOGICAL AND BEHAVIORAL COMPARISONS. George V. Rebec and David S. Segal. Dept. Psychiat., Sch. Med., UCSD, La Jolla, CA. 92093.

Systemic administration of d-amphetamine, but not methylphenidate, in rats produces a dose-dependent decrease in dopamine (DA) biosynthesis in the neostriatum and nucleus accumbens. To determine if this difference reflects a differential activation of DA receptors, we monitored d-amphetamine- and methylphenidate-induced changes in the spontaneous activity of single neurons in the caudate-putamen of immobilized, locally anesthetized rats. Both drugs acted similarly, producing an initial potentiation of firing rate followed by a marked depression of unit activity. These results indicate that the amphetamine-induced suppression of DA biosynthesis is not due to receptor-mediated feedback mechanisms.

Previous behavioral evidence indicates that the response to d-amphetamine, but not methylphenidate, is antagonized by α-methylparatyrosine (αMT) pretreatment, while reserpine selectively attenuates the methylphenidate response. Thus, DA biosynthesis may be specifically responsive to an αMT-sensitive pool. In our behavioral experiments, however, we have found that the response to d-amphetamine (2.5 mg/kg) and methylphenidate (25 mg/kg), to a lesser extent, is significantly reduced by αMT pretreatment (150 mg/kg, 2 hr before). Reserpine pretreatment (2.5 mg/kg, 2 hr before) was also found to attenuate the response to both stimulants, although the effect on methylphenidate-induced behaviors was more pronounced. Our results indicate that since behavioral effects produced by amphetamine and methylphenidate appear to be mediated by release from both a "newly synthesized" and "storage" pool, the amphetamine-induced suppression of DA biosynthesis cannot be simply explained by the selective action of this drug on newly synthesized catecholamines.

1272 THE COMPARATIVE BEHAVIORAL EFFECTS OF CHRONIC ADMINISTRATION OF d-AMPHETAMINE, IMIPRAMINE, & PEMOLINE TO SELECTED MEMBERS OF A JUVENILE PRIMATE SOCIAL COLONY. R F Schlemmer, Jr, R C Casper*, F K Siemsen*, D L Garver, & J M Davis. Ill State Psychiatric Inst & U of Ill Med Center, Chicago, IL 60612.

Although varying in chemical structure & in pharmacology, d-amphetamine (d-A), imipramine (Imip), & pemoline (Pem) are all recognized as effective agents in the treatment of the hyperkinetic child syndrome. We have previously reported that chronic administration of d-A to members of a juvenile primate social colony results in significant behavioral changes. Since d-A is known to affect both dopamine systems (DA) & norepinephrine systems (NE); Imip, an agent that preferentially affects NE, & Pem, an agent that preferentially affects DA, were tested in an attempt to evaluate the relative contribution of DA & NE in this syndrome. In the expts, each drug was administered n.g. daily to 3 members of a stable peer-raised, juvenile Stumptail macaque social colony of 6 animals. Behavioral observation by a "blind" observer occurred for 76 mins on designated AMs during baseline & treatment periods in each expt. Drugs were administered as follows: d-A SO_4 0.5 mg/kg for 4 wks, Imip HCl 3.33 mg/kg for 3 wks, & Pem 2.5 mg/kg for 5 wks. d-A, Imip, & Pem all significantly decreased play activity, but significantly increased social grooming from baseline levels in treated monkeys. d-A & Imip, but not Pem significantly increased huddling with eyes open. d-A & Pem induced stereotyped behavior, but Imip did not. Unless these 3 agents possess an undetermined common mechanism of action, these results suggest that both DA & NE may independently mediate the decreased play & increased social grooming seen in the treated juvenile monkeys; however, the increased huddling with eyes open appears to be modulated by NE, while stereotypy appears to be modulated by DA.

1273 EFFECT OF CHRONIC ADMINISTRATION OF d-AMPHETAMINE AND HALOPERIDOL IN d-AMPHETAMINE MORTALITY IN RATS. Henry L. Schreiber, Robert W. Bell, Michael E. Kufner*, Linda L. Wright* and Ramiro Villescas*. Dept. of Psychol., Texas Tech University, Lubbock, Tx. 79409.

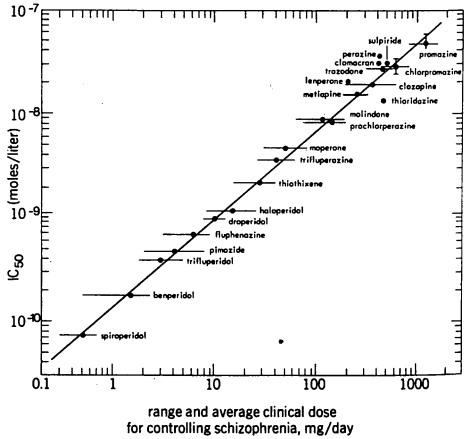
The present study determined whether chronic administration of d-amphetamine, chronic administration of haloperidol, or both, would affect mortality to a high dose of d-amphetamine (100 mg/Kg, i.p.). Thirty male Sprague/Dawley rats received d-amphetamine (2.5 mg/Kg, i.p.) and 30 male Sprague/Dawley rats received a comparable volume of saline for 8 consecutive days, 30±5 min prior to a 2 min test session in a Y-maze. Then, d-amphetamine and saline groups were divided in half to form 4 groups: 15 subjects received haloperidol (.5 mg/Kg, s.c.) 90±10 min prior to amphetamine injection (2.5 mg/Kg)(Hal/am group); 15 subjects received sterile water (comparable ml, s.c.) prior to amphetamine injection (Wat/am group); 15 subjects received haloperidol prior to saline injection (Hal/sal group); and 15 subjects received water prior to saline injection (Wat/sal group). Subjects were tested, as before, for a 2 min test session 30±5 min after amphetamine or saline injection for 6 days. On Day 15, all subjects received Wat/sal injections prior to testing. On Day 16, all subjects received d-amphetamine (100 mg/Kg). During the d-amphetamine injection period, amphetamine subjects showed a reciprocal pattern of increasing stereotypy and decreasing rearings and entries into the arms of the Y-maze. Saline subjects showed no such pattern. During the haloperidol injection period, Wat/am subjects continued to show increasing stereotypy, whereas, Hal/am subjects showed decreasing stereotypy. On Day 16, subjects which had received d-amphetamine (2.5 mg/Kg) showed increased latency to death, regardless of whether they had received haloperidol or water pretreatment.

1274 CORRELATION OF ANTI-PSYCHOTIC DRUG POTENCY AND NEUROLEPTIC RECEPTOR BLOCK. P. Seeman, T. Lee, M. Chau-Wong and K. Wong. Pharmacology Department, University of Toronto, Toronto, Canada.

In order to test whether all clinically effective antipsychotic drugs block neuroleptic and dopamine receptors, a wide variety of neuroleptic drugs were tested on the stereospecific binding of ^{3}H-haloperidol or ^{3}H-dopamine to calf caudate homogenate. Modifying the method of Seeman et al. (Proc. Nat. Acad. Sci., 72, 4376, 1975), the final mixtures were filtered through GF/B filters and washed; stereospecific binding was that amount of isotope bound in the presence of (-)-butaclamol minus that in the presence of (+)-butaclamol (active form). The adjacent Fig. indicates that there is an excellent correlation between the antipsychotic potencies and the concentrations which inhibit ^{3}H-haloperidol binding by 50%.

The $IC_{50\%}$ values may also be of therapeutic value since they are similar to the concentrations of these drugs in the treated patient's plasma water.

(Supported by Ontario Mental Health Foundation and the M.R.C. of Canada MT-2951).



1275 SENSITIZATION TO STIMULATION BY COCAINE IN MICE. Louis Shuster, Dept. of Biochem. and Pharmacol., Tufts Univ. Sch. of Med., Boston, Ma.,02111 USA

The running response of B_6AF_1/J mice to a test dose of 20 mg/kg cocaine·HCl was increased 4-fold by pretreatment with 4 daily injections of the same dose of cocaine. This sensitization persisted for as long as 2 months. Cocaine-pretreated mice did not show an increased running response to either morphine or d-amphetamine. The response to cocaine was increased 2-fold by pretreatment with morphine, and 3-fold by pretreatment with amphetamine. Pretreatment with reserpine or imipramine had no effect. Cocaine pretreatment did not alter whole-brain levels of norepinephrine or dopamine. A single injection of 20 mg/kg cocaine decreased the in vivo conversion of tritiated tyrosine to catecholamines in the brains of control mice, but had no effect to cocaine-pretreated mice. Sensitization to cocaine may be associated with an alteration in the regulation of catecholamine turnover in the brain.

When different strains of mice were tested, there were marked genetic differences in both the initial response to cocaine and in the extent of sensitization after repeated injections. The genetic determinants that control the acute response to cocaine are different from those involved in sensitization.

1276 EFFECT OF TWO NON-ANTIPSYCHOTIC BUTYROPHENONES ON DOPAMINE AND ITS METABOLITES IN THE STRIATUM AND TUBERCULUM OLFACTORIUM OF THE RAT. M. Stanley* and S. Wilk* (Spon: J.P. Green). Mt. Sinai Sch. of Med., N.Y., N.Y. 10029

The effects of two butyrophenones, AL-499 and AHR-1900, on dopamine (DA) and its metabolites in the striatum and tuberculum olfactorium (TO) of the rat were compared with effects seen following the classic antipsychotic butyrophenone haloperidol. Because of favorable results in animal screening tests, AL-499 and AHR-1900 underwent clinical trials in chronic schizophrenia and were found to be devoid of any antipsychotic properties at dosages of 600 and 800 mg/day respectively. AL-499 subsequently studied at doses as high as 1200 mg/day revealed only weak antipsychotic activity. In both cases the doses employed were well in excess of those typically used in this potent drug class. All drugs were injected ip and the rats were decapitated one hour later. The striatum and TO were separately homogenized in cold 1N HCl. The major metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed by gas chromatography. Dopamine was determined by a new gas chromatographic method employing pentafluoropropionic anhydride as the derivatizing reagent and α-methyl DA as internal standard. None of the butyrophenones had any effect on striatal DA levels. AL-499 had no effect on DOPAC and HVA levels in striatum and TO in the dose range studied (1-40 mg/kg). AHR-1900 significantly elevated DOPAC and HVA in both regions but the dose-response curve was atypically flat. Moreover, its ED50 for elevation of striatal DOPAC exceeded that of haloperidol (0.15 mg/kg) by a factor of 65. These results suggest that compounds which do not cause a substantial increase in DA turnover in rat striatum will not be effective antipsychotics and that elevation of striatal DA metabolites is a good predictor of antipsychotic efficacy. These experiments further strengthen the hypothesis that DA is involved in the pathophysiology of schizophrenia. (Supported by MH-21638.)

1277 DMT INDUCED TURNING IN UNILATERAL NIGRAL LESIONED RATS: EVIDENCE FOR CENTRAL DOPAMINERGIC ACTIVATION. Warren C. Stern and Mario Dalsass. Squibb Inst. Med. Res., Princeton, N. J. 08540.

Rats with unilateral lesions of the substantia nigra exhibit rotational behavior following pharmacological administration of dopaminergic (DA) agonists. This is believed to result from assymetric activation of the nigro-striatal DA system. Employing this turning model as an index of brain DA receptor activity we examined (a) the effects of N,N-dimethyltryptamine (DMT), an "endogenous psychotogen" implicated in the etiology of schizophrenia, and (b) the ability of neuroleptics to block the effects of DMT. The results showed that approximately 50% of rats with unilateral electolytic or 6-hydroxydopamine lesions of the substantia nigra gave significant increases in rotational behavior after 10 or 20 mg/kg, i.p. of DMT (90-100% of the rats rotated after d-amphetamine injection). Two neuroleptics, clozapine (0.1-2.0 mg/kg, i.p.) and pimozide (0.05-0.1 mg/kg, i.p.) totally blocked the DMT induced rotation. Control agents, Librium (10 mg/kg), α-methyltyrosine (100 mg/kg) and methysergide (5 mg/kg) did not reduce DMT-turning. The present nigral-turning results suggest that DMT has apomorphine-like stimulating effects on central DA receptors, effects which can be blocked by low doses of neuroleptics. Two major neurochemical hypotheses of schizophrenia, i.e. the DMT-endogenous hallucinogen and the DA hyperactivity views, may represent two facets of the same physiological process, namely, the stimulation of DA receptors by DMT.

1278 AN ANIMAL BEHAVIOR MODEL OF THE ACTIONS OF LSD: DURATION OF BEHAVIORAL EFFECTS AND ONSET AND DURATION OF TOLERANCE. Michael E. Trulson and Barry L. Jacobs, Dept. Psychol., Princeton Univ., Princeton, N.J. 08540

Two of the most impressive behavioral effects of LSD in humans are the long duration of action and the dramatic tolerance which develops following its repeated administration. Utilizing the behavioral syndrome described in the preceding abstract, we investigated these parameters of the action of LSD in cats. Cats displayed limb flicking and abortive grooming behaviors significantly above baseline levels up to 8 hrs following a dose of 50ug/kg of LSD and 4 hrs after 10ug/kg of LSD. Tolerance to a test dose of 50ug/kg of LSD begins to develop within 2 hours (46% below baseline) following a single dose of 10ug/kg of LSD. Tolerance is very marked (77% below baseline) at 6 hrs and virtually complete (92% below baseline) at 24 hrs post-injection. A substantial tolerance is still present three days (47% below baseline) post-injection, but is not statistically significant 5 days post-injection. By comparison, the tolerance to a test dose of 50ug/kg of LSD following a single dose of 50ug/kg of LSD is more pronounced and of longer duration. Tolerance under these conditions is complete at one day post-injection, and a substantial tolerance (80% below baseline) is present at 3 days post-injection. A large, statistically significant, tolerance (40% below baseline) is still present 5 days post-injection. Tolerance was also observed utilizing more tradition measures of LSD's action such as play and overt hallucinatory behavior.

1279 ATTENUATION OF THE EXTINCTION OF LATERAL HYPOTHALAMIC SELF-STIMULATION BEHAVIOR BY APOMORPHINE, D-AMPHETAMINE AND METHYLPHENIDATE. Richard A. Vogel, T. Steven Barlow*, and George R. Breese. Dept. Psychiatry, U.N.C. School of Medicine, Chapel Hill, N. C. 27514.

Rats were implanted with bipolar electrodes aimed at the lateral hypothalamus (LH) and were trained to press a lever at moderate to high rates for electrical stimulation on a CRF schedule. Daily sessions consisted of 3 periods: 15 min of responding with electrical reinforcement (current on) followed by a drug or saline injection; 15 min of responding with the current on; and at least 30 min of extinction (current off). During control sessions response rates remained stable for the first 30 min and rapidly approached zero within the first 5 min of extinction. While apomorphine (0.2 mg/kg) decreased reinforced responding 50%, responding was dramatically increased (850%) during the first 30 min of extinction in 14 of 15 rats. This finding is similar to that of Broekkamp and van Rossum (Psychopharmacologia, 34:71, 1974). Like apomorphine, d-amphetamine (3 mg/kg) decreased reinforced responding (25%) and increased responding during the first 30 min of extinction (3000%) in 8 of 10 rats. A 2 mg/kg dose of d-amphetamine produced this effect in only 1 of 7 rats. Methylphenidate (30 mg/kg) similarly decreased reinforced responding 50% while greatly increasing responding during the first 30 min of extinction (4500%) in 8 of 10 rats. Although response rates during extinction were lower than reinforced response rates after all drugs, the duration of bar press responding during extinction in drug treated rats ranged from 15 to 200 min. These findings suggest two possibilities: (1) intense dopaminergic stimulation is discriminated as equivalent to LH stimulation; and/or (2) the morphology of drug-induced stereotyped behavior can be conditioned by electrical stimulation of the brain and can remain extremely stable after its termination. (Supported by USPHS Grants MH-16522, HD-03110 and MH-25573).

1280 THE ACUTE METHADONE DRUG OVERLAY ON MONKEY EEG. T. Joe Willey. Dept. Physiol. Pharm. Sch. Med. Loma Linda Univ. Loma Linda CA. 92354

The drug effects of acute methadone administration into the venous pool was studied in the monkey, Macaca nemestrina. The subjects were prepared for chronic experiments with an indwelling catheter in the internal jugular vein and surface and depth probes over cortical tissue or in various mesolimbic subcortical nuclei. Pre- and postdrug spontaneous electrical signals were obtained using a miniaturized head-mounted 12 channel PAM/FM biotelemeter. The data was recorded by conventional methods and stored on analog magnetic tape. EEG and EOG analysis was carried out using a computer with the data processing objectives to reduce signal dimensionality and evaluate the drug effects. Frequency spectra were computed for the EEG and plotted in a time series profile to reveal trends related to the drug effects. The EOG activity index was computed by integrating deflections during eye motions. Ten days elapsed between test doses given according to a latin square design.

The effects of methadone on brain functions are variable and complex. At low doses (ie. 0.25 - 0.50 mg/kg) the EEG is altered from normal "scanning" conditions to a lower amplitude higher frequency phase featuring no intermittent burst activity for several minutes. The next phase retains similiar patterns but include episodes of EEG slow wave higher amplitude activity terminated by two second rhythmic bursts. At higher doses (1 - 2 mg/kg) these two phases are sometimes preceded by eliptiform patterns lasting about 2 min. The onset, duration and sequencing of these phases are dose dependent. The EOG index is suppressed during the drug overlay and also is dose dependent. At 4mg/kg there is a strong tendency for respiratory depression in the monkey and concomitant EEG and EOG isoelectric effects. (Supported by NIDA 00721)

1281 CONCURRENT INTRACRANIAL SELF-STIMULATION AND AMPHETAMINE SELF-ADMINISTRATION IN RATS. Roy A. Wise and Robert A. Yokel. Center for Research on Drug Dependence, Psychology Department, Concordia University, Montreal.

Rats were implanted with lateral hypothalamic electrodes and trained to lever-press for 25-30 ua sine-wave stimulation. They were then fitted with intravenous catheters, and tested for concurrent self-stimulation and d-amphetamine self-administration (.25 mg/kg/injection) in a two lever choice situation. Animals settled into stable patterns of responding, showing normal rates of self-administration with sometimes slower and sometimes faster than normal rates of self-stimulation. Self-stimulation was continuous, except for half-hourly pauses for amphetamine responses, and except for periods when lever-reward contingencies were switched where unusual drug levels were taken before adjustments were made. In the latter case self-stimulation abated in proportion to the excess drug taken. Dopamine receptor blockade with [+]-butaclamol (.05 mg/kg) caused decreased self-stimulation and increased self-administration as is seen when the drug is tested with each response alone.

These preliminary data have three interesting implications. First, they make it clear that the rate of amphetamine responding is not normally limited by stereotypy or some other high-dose, incapacitating effect of amphetamine: animals showed the capacity for continuous lever-pressing between amphetamine responses. Second, it is clear that the facilitation of self-stimulation by amphetamine can accompany doses that are sufficient to be rewarding in themselves. Why should amphetamine reward cause increased work output for stimulation reward, especially when the same mechanism is thought to be activated by both rewards? Third, our data rule out the possibility that dopamine receptor blockade inhibits self-stimulation simply by interfering with some aspect of response capacity. The inhibited self-stimulation coincided with the period of accelerated self-administration after butaclamol treatment.

Sleep

1282 VIGILANCE: AN IMPORTANT DETERMINANT OF CORTICAL EYE MOVEMENT POTENTIALS

Robert M. Bowker *and Adrian R. Morrison. Laboratories of Anatomy, School of Veterinary Medicine, University of Pennsylvania, Phila. 19174.

A major criterion for distinguishing cortical eye movement potentials (EMP), or PGO spikes of wakefulness, from spontaneous PGO spikes during paradoxical sleep,(PS),is that the former disappear or become greatly reduced in darkness (2,3). Recent studies in our laboratory have indicated that the level of illumination is of far less importance than the degree of alertness of the animal.

Six cats were implanted with routine EEG, EOG, and EMG electrodes, as well as transcortical electrodes in the visual cortex. Recordings were made in light and darkness while the cat resided in a sleep chamber with an observation window.

When placed in the lighted chamber, cats explored the cage and gradually acclimated to the recording situation, usually within 15-20 minutes. Head and eye movements were especially plentiful as the cat actively explored the chamber. Each eye movement was accompanied by a cortical EMP as others have reported (2,3). With acclimatization the cat often assumed a sphinx posture. Although cortical EMP continued to accompany each eye movement, the amplitude was attenuated in contrast to those occurring during active visual exploration and during PS in the same cat. The EEG during this period of quiet wakefulness, however, remained desynchronized. If a novel visual (experimenter's hand), sound (3000 hz tone burst) or olfactory stimulus was presented, which startled the cat or to which it oriented, the EMP returned to an amplitude equalling that recorded in PS.

In the dark the cat behaved exactly as in the lighted condition. EMP gradually became attenuated and often disappeared; but eye movements persisted, in confirmation of other reports (2,3). However, if a visual, auditory or olfactory stimulus, which elicited a startle or an orientation response, was introduced, the EMP reappeared and were of equal amplitude to the spontaneous spikes of PS (fig. 1). The amplitude of the EMP gradually decreased again, usually within 10-20 seconds, if no new stimuli were presented.

These results indicate that the appearance of EMP is very much dependent upon a state of active alertness (startle or orientation responses) and that the diminution in amplitude of EMP of wakefulness stems primarily from a reduction in vigilance of the cat. Furthermore, the fact that novel and startling stimuli elicit EMP during wakefulness and PGO spikes during sleep (1) equal in amplitude to spontaneous PGO spikes, irregardless of lighting conditions, supports the hypothesis that the classical PGO spike of PS is merely a sign of activation of the startle reflex network.

Supported by N.I.H. Grant MH15767

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2. Brooks, D.C. and Gershon, M.D., Brain Res., 27/223-239, 1971.
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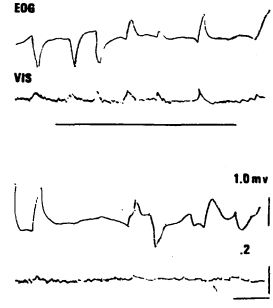

Figure 1.:
Continuous recording of a normal cat in darkness with presentation (solid line) and removal (below) of visual stimulus to which the cat oriented. Identical results were obtained with auditory and olfactory stimuli. EOG-electro-oculogram; VIS-visual cortex; Time calib- 1 sec.

1283 PGO SPIKES IN THE LOCUS COERULEUS OF THE ALBINO RAT? J. Farber*, G. Marks*, M. Rubinstein* and H. Roffwarg. Dept. Psychiat., Montefiore Hospital and Medical Center and Albert Einstein College of Medicine, Bronx, N.Y. 10467.

Several studies have failed to find PGO spikes in REMS in the albino rat in brain sites (LGN and Vis Cx) where they are readily obtainable in the cat (Stern, W. et al, Physiol. Behav. 12: 293, 1974). On the other hand, Gottesman (Physiol. Behav. 4: 495, 1969) reported observing spike waveforms from the oculomotor nuclei and "parasagittal" pons in the albino during REMS, though reserpine seemed not to alter the distribution of these subcortical waves as with PGO spikes in the cat. Cespuglio et al (APSS, 1975) have also reported finding PGO-type waves during REMS, but in the VI and III nerve nuclei in agouti rats, that coincided with phasic activity in the extra-ocular muscles and vibrissae. However no activity of this kind could be recorded in REMS in the area of the LGN in chronic and in reserpine- or PCPA-pretreated preparations. The latter only when curarized, showed central phasic activity at the border of the dorsal LGN.

We have initiated a central phasic mapping study of the albino rat's hindbrain in an attempt at cross-species verification of the PGO spike. Bipolar stainless steel electrodes (0.5 mm between the tips) are aimed at the nuclei of the locus coeruleus and subcoeruleus, the motor nuclei of the IIIrd and VIth, the Vth and VIIth nerves (associated with MEMA in the cat), and other hindbrain nuclei. In addition, the animals are implanted with EEG, EMG and EOG electrodes.

Results: We find that sharp waves (25-60uv amplitude, 80-100 msec duration) that are clearly associated with REM sleep can be recorded from the dorsolateral pons. Histological examination has revealed that the site from which this activity is recorded is the area of the locus coeruleus.

85% of total spike activity occurs in REMS, 6.6% in the two minutes of SWS preceding REMS onsets, and 6.5% at points in SWS that precede spontaneous arousals. Their average frequency in REMS is approximately 25 spikes per min. A serial increase in spike frequency occurs in the first few 30 sec epochs of REMS. An association of the pontine spiking and ocular movement was found in REMS. The burst pattern of the sharp waves is seen only in this stage. The probability that a single eye movement and a single spike are simultaneous is .35, whereas for a burst of spikes, it is .87. This is reminiscent of Pompeiano's distinction between Type I and Type II PGO waves in the cat. Under reserpine (3mg/kg), the pontine spikes we observed lost their REMS specificity, became aperiodic, and were observed also with synchronized and desynchronized EEGs.

In summary, we report a pontine sharp wave that in its electrophysiological characteristics and distribution corresponds to the pontine PGO wave of the cat. This is the first demonstration of PGO-type activity in the rat in a pontine area (locus coeruleus) other than a motor nucleus.

1284 ACROSS-NIGHT TRENDS IN HUMAN SLEEP CYCLE DURATIONS, I. Feinberg and T.C. Floyd, VA Hospital, San Francisco, CA.

It is frequently asserted that sleep cycle durations vary randomly around a mean of 90 min. It is customary to describe REM cycles (REMCs), the amount of sleep elapsed between successive REM episodes ($REMP_1$ + $NREMP_2$.. etc.). However, defining cycles in this way omits the first episode of NREM sleep. Since this episode contains 50% of the stage 4 activity of the night and is highly sensitive to age, we prefer to measure NREM sleep cycles (NREMCs), which are measured from successive NREM onsets ($NREM_1$+ REM_1 ...etc.). Figs. 1 & 2 show that both REMCs and NREMCs exhibit statistically significant curvilinear trends across the night. These trends are directly predictable from the successive durations of REM and NREM episodes considered separately (1,2).

Changes in sleep characteristics across the night may provide clues to the kinetics of the metabolic processes which underlie the sleep EEG. If the function of sleep is to reverse the effects of wakefulness on the brain, this process ought not occur at a constant rate; instead, the rate should be a function of the amount of substrate accumulated. This should be determined by the duration and intensity of waking brain activity. Some aspects of NREM sleep fit this pattern. Thus, stage 4 EEG is a function of the duration of waking (3). The decline in stage 4 with age (1) may reflect decreasing intensity of waking brain activity. Stage 4 activity declines sharply across the night when stage 4 is depleted, NREM sleep episodes (now made up of Stage 2 sleep) become shorter.

REM sleep, rather than being related to the duration of preceding wakefulness, is a positive function of the duration of sleep itself. MAO inhibitors can eliminate REM sleep in man with no deleterious effects. This and other considerations led us to suggest that REM sleep may occur cyclically in order to provide a substrate needed to permit the optimal occurrence of NREM sleep. This model, which is described in detail elsewhere (2), integrates the following observations on human sleep: the alternation of NREM and REM episodes, the decrease in stage 4 with age, the relation of stage 4 to prior wakefulness, the decline in stage 4 across the night, and the decline in NREM durations when stage 4 is depleted.

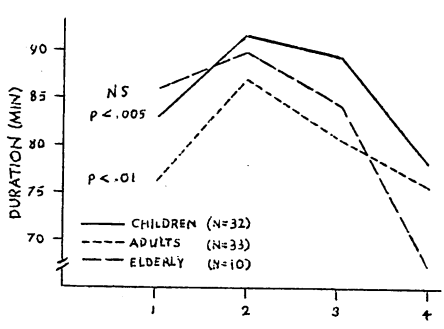

FIG. 1. REM SLEEP CYCLE (REMC)

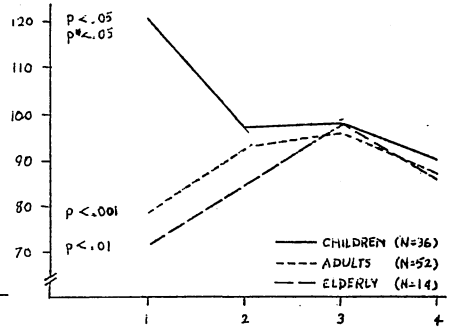

FIG. 2. NREM SLEEP CYCLE (NREMC)

*Significant cubic component. All other ps shown in both figs. represent significant quadratic components.

(1) Feinberg, I., Koresko, R.L., & Heller, N., J Psychiat. Res. (1967), 5:197; (2) Feinberg, I., J. Psychiat Res., 1974., 1974, 10:283; (3) Webb, W.B. & Agnew, H.W. Jr., Science, 1971, 174:1354

1285 TWO TYPES OF HIPPOCAMPAL RHYTHMICAL SLOW ACTIVITY AND NEOCORTICAL LOW VOLTAGE FAST ACTIVITY DURING ACTIVE (REM) SLEEP IN THE RAT. Terry E. Robinson*, Ronald C. Kramis* and C.H. Vanderwolf. Dept. Psychology, University of Western Ontario, London, Canada.

Recent studies (Kramis et al., Exp. Neurol., 1975, 49, 58-65; Vanderwolf, J. comp. Physiol. Psychol., 1975, 88, 300-323) suggest that the hippocampus receives 2 non-specific inputs from the brainstem, each capable of producing rhythmical slow activity (RSA), and that the neocortex receives 2 similar inputs, each capable of producing low voltage fast activity (LVFA). An atropine-sensitive input produces RSA (usually 4-7 Hz) and LVFA which is abolished by atropine sulfate but is not abolished by volatile anesthetics or urethane. Activity in this system is not consistently related to concurrent motor activity. A second input produces RSA (usually 7-12 Hz) and LVFA which is not abolished by atropine sulfate but such RSA is abolished by volatile anesthetics or urethane and such LVFA by volatile anesthetics. Activity in this atropine-resistant system is closely related to concurrent "voluntary" movement. That is, atropine-resistant RSA and LVFA occur only during the performance of behaviors such as walking, running, swimming and jumping but do not occur during immobility or behaviors such as facewashing, chewing or licking. These data were obtained from waking animals. The present experiment was designed to determine if the RSA and LVFA which occur during active sleep resembles that of the waking state.

Seventeen rats were implanted with bipolar recording electrodes in the neocortex and hippocampus. In some rats electrodes to record EMG were placed in the nuchal muscles and under the vibrissal pads. Rats were deprived of active sleep for 3-5 days using the inverted flowerpot technique. Recordings were then taken after the following treatments: 1) CONTROL: a) undrugged, no sleep deprivation, b) undrugged + active sleep deprivation, c) atropine methyl nitrate (40 mg/kg) + active sleep deprivation; 2) ATROPINE SULFATE (40 mg/kg) + active sleep deprivation; 3) URETHANE (1-1.5 g/kg) + active sleep deprivation; and 4) URETHANE (1-1.25 g/kg), no sleep deprivation.

Control animals with appropriate recording placements show nearly continuous RSA and LVFA during active sleep. The mean frequency of RSA is 8.0 Hz during twitch (phasic) periods and 6.9 Hz during non-twitch (tonic) periods. After treatment with atropine sulfate active sleep still occurs and RSA and LVFA persist during twitch periods. However, virtually all the RSA and LVFA normally seen during non-twitch periods is abolished. After atropine only very high frequency RSA ($\bar{X}$ = 9.6 Hz) was observed. In sleep deprived rats given urethane active sleep also continued to occur, but 8-12 Hz RSA was not observed and episodes of muscular twitching were rare. During the active sleep periods only low frequency ($\bar{X}$ = 6.2 Hz) RSA occurred. LVFA was common in the neocortex but spindle activity was more frequent than in the control condition.

The results show that both atropine-resistant (anesthetic-sensitive) and atropine-sensitive (anesthetic-resistant) forms of hippocampal RSA and neocortical LVFA are present during active sleep. In addition, the atropine-resistant cerebral activation, which is related to ongoing motor activity during waking, is also related to motor activity (twitches) during active sleep. The reduced motor activity seen in active sleep as compared to vigorous waking behavior may be due to descending inhibition of ventral horn cells and Ia afferent terminals, as suggested by Pompeiano (Res. Publ. Ass. nerv. ment. Dis., 1967, 45, 351-423; also see Whishaw and Vanderwolf, Behav. Biol., 1973, 8, 461-484).

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1286 THE RECEPTOR THEORY OF ANESTHESIA. H. C. Sabelli, H. S. Havdala*, B. Diamond*, and J. May*. Mt. Sinai Hosp., Chicago, Ill. 60608 and Chicago Med. Sch., Chicago, Ill. 60612.

Anesthesia is attributed to physical interactions with nonfunctional tissue constituents (lipids, water), resulting in generalized depression of excitability; such "membrane stabilization" is considered as the common mechanism for both general (GA) and local anesthetics (Seeman, Pharmacol. Rev., 1972). We propose that anesthesia results from the alteration in the function of structurally specific moieties of membrane biopolymers responsible for chemically-triggered changes in membrane enzymatic activity and potential. The alteration of such "receptors" for neurotransmitters would lead to selective changes in synaptic activity (anesthesia). Evidence: (1) The presence of a multiplicity of neurotransmitter receptors in frog nerves (Sabelli and Gorosito, Int. J. Neuropharmacol., 1969) provided us with an opportunity to examine in vitro the interactions of GA and local anesthetics with putative neurotransmitters. Using external electrodes for stimulation and recording of frog sciatic action potentials, we have found: (a) differential interactions with catecholamines (CA): Halothane axonal effects appear to involve β-adrenergic receptors because its spike amplitude-lowering effect is augmented by epinephrine and isoproterenol and prevented by sotalol. Similarly, pentobarbital is potentiated by norepinephrine and by epinephrine and antagonized by propranolol and by sotalol. In contrast, the axonal effects of the convulsant barbiturate DMBB [5-ethyl-(1,3 dimethylbutyl) barbituric acid] and of the anticonvulsant phenobarbital are inconsistently modified by CA, and those of procaine are antagonized by α and β CA and enhanced by α and β blockers. (b) differential interactions with gamma amino butyric acid (GABA): GABA accentuates the actions of halothane, phenobarbital and procaine while decreasing those of pentobarbital and DMBB. (c) Similar differential interactions with GA and local anesthetics, different types of barbiturates, tranquilizers, etc. are observed with histamine, serotonin, muscarinic and nicotinic cholinergic agents, with the corresponding specific receptor blockers, with cyclic adenosine monophosphate and guanosine monophosphate, and with Ca^{++}, at variance with the view that these agents share a common "stabilizing" action on excitable membranes. (2) The microiontophoresis of procaine to frog nerves (in vitro) or to corpus callosum fibers (gallamine-paralyzed rabbit) induces transient firing (which parallels its initial irritant effect) followed by local anesthesia; such biphasic effect, excitation followed by inhibition, is characteristic for partial agonists acting upon receptors (e.g. succinylcholine in the neuromuscular junction). (3) During hypnosis induced by chloralose or by barbiturates in rabbits, cortical evoked potentials are enhanced or not altered, indicating that some synaptic pathways are not depressed. (4) Anesthesia can be produced by drugs which are most likely receptor specific (e.g. fentanyl, ketamine). (5) As with receptor acting drugs, minor changes in the structure of GA produce marked changes in biological activity (e.g. convulsions by flurothyl or by DMBB). (6) The convulsant action of local anesthetics can be prevented by some derivatives suggesting competition for receptors. (R.K. Richards, in Chemical Modulation of Brain Function, Raven Press, 1973). (7) The interactions between GA and CA in heart and lung (Klide et al., Anesth. and Anal., 1969) indicate that GA modify adrenergic receptors. (8) GA and barbiturates selectively enhance or inhibit the neuronal effects of microiontophoretically administered neurotransmitters (Roberts and Straughan, J. Physiol. 1967; Salmoiraghi & Weight, Anesthesiol., 1967).

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1287 BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF SLEEP PERFUSATE SOLUTES FROM CATS. Curt W. Spanis, Ma del Carmen Gutiérrez* and René R. Drucker-Colín. Univ. of San Diego, San Diego, Ca., and Instituto de Biologia, Universidad National Autonoma de Mexico, Mexico 20, D.F.

Proteins have been isolated, from sleep inducing perfusates obtained from the MRF of conscious cats during REM sleep, by means of push-pull cannulae (Science 187:963-965, 1975). These experiments describe further biochemical and physiological characterizations of these solutes and correlate them with behavioral states of the animals. Solutes from REM and awake perfusates were concentrated initially by diaflo ultrafiltration. Small peptides were desalted on G10 sephadex columns and concentrated by lyophilization. A variety of biochemical assays on the extracted solutes demonstrated several new findings. By sodium dodecyl sulphate slab gel electrophoresis, two polypeptides were isolated from REM perfusates which were not observed in awake solutes. These were estimated to be of mol. wts. 73,000 and 45,000. However, separation of REM and awake solutes by isoelectric focusing on polyacrylamide gels demonstrated the presence of three macromolecules in REM perfusates not seen in awake samples. Also by this method, pH gradients for REM solutes showed that these polypeptides were predominantely acidic as compared to those in CSF and serum. Gas chromatographic analysis of the solutes revealed a fivefold increase in galactosamine in REM solutes over awake solutes. Further, examination of REM and awake solute hydrolysates with an amino acid analyzer revealed two things: one, concentrations of several amino acids differed considerably between the two samples, the samples being equilibrated for equal protein concentration prior to hyrolysis, and two, one additional amino acid was found in the REM solute hydrolysate. The number of free polypeptides under mol. wt. 10,000 was negligible in unconcentrated and tenfold concentrated perfusate solutes. Perfusate solutes differed significantly in solute concentrations and types of macromolecules from those in serum and CSF, indicating that the latter do not appear to contribute to the solutes in the perfusates, in agreement with our earlier supportive data (op.cit.). Further, the source of perfusate solutes was traced by introducing labelled amino acid into cannulae implanted in the third ventricle and PO areas. Subsequent label appearing in MRF perfusates was negligible which further supports the concept that CSF does not contribute, at least directly, to perfusate solutes. Also, lesioning in the PO area resulted in disruption of the cyclic release of proteins which normally accompany major bouts of REM sleep as reported earlier (op.cit.). Finally, in studies now initiated, the onset of SW and REM sleep in alert cats appears to be shortened and the duration and frequency of these cycles increased following 'push-pull' perfusion of concentrated REM solutes into the MRF of the animals. The injected solutes ranged from 1,000 to 100,000 daltons as prepared with molecular ultrafilters. It is suggested that moderately large polypeptides may participate in the regulation of REM sleep.

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1288 MODIFICATION OF MASSETERIC REFLEX ACTIVITY DURING SLEEP AND WAKEFULNESS BY STIMULATION OF THE PONTO-MEDULLARY JUNCTION IN CATS. Margaret I. Babb*, Nancy K. Wills-Lewis* and Michael H. Chase. Depts. Physiol. and Anat., Sch. Med., UCLA, Los Angeles, CA. 90024.

Regions within the caudal pons and medulla are generally considered to play an inhibitory role in the modulation of motor activity. The purpose of this study was to assess this role by stimulating sites at the level of the ponto-medullary junction and examining the resultant changes in an evoked masseteric reflex during states of sleep and wakefulness. EEG, EOG and neck EMG electrodes were implanted for monitoring sleep and waking states in eight chronic cats. Bipolar electrodes were implanted in the mesencephalic V nucleus for evocation of the masseteric reflex which was monitored by wire loop electrodes fixed in the masseter muscle. Bulbar sites were stimulated by means of bipolar strut electrodes (3 pulses/400 cps). Conditioning (bulbar) - test (masseteric reflex) latencies were systematically explored from 5 to 100 msec.

Stimulation of the ponto-medullary region of the brain stem strongly affected motor activity during quiet and active sleep as well as during wakefulness. The predominant response was early inhibition (<15 msec) throughout all states. From 15 to 40 msec an emergent pattern of facilitation occurred during wakefulness and quiet sleep, while during active sleep the reflex was inhibited. Sites that most reliably produced the early inhibition were located in the ventral and medial portions of the brain stem.

1289 SLEEP EEG DATA MANAGEMENT AND ANALYSIS PACKAGE (SLEEPANA). David Bern* and William Fishbein. Dept. Psych., City College of CUNY, New York, N.Y. 10031.

A major problem in performing long-term sleep/chronobiological research is the difficulty and tedium of reducing and assembling the enormous amount of physiological data into more usable form. This computer package provides rapid processing of EEG/EMG data after being translated into numerical form. Data are easily managed and analyzed by various program modules of the system. The first module is a conversational program that enables the user to easily type data into the computer with minimum concern for typographical errors. The second conversational program allows the user to easily correct, modify or update data stored in the computer. Reduction of the data is then performed by a third program module that provides a list of errors encountered in the raw data. Once the raw data have been "cleaned up," various statistical analyses and tests can be performed using a standard statistical program package. This package provides information to a fourth module that generates graphs that summarize the data. A salient feature of the system is the ability to closely monitor the data in its various stages and its flow from one module to the next.

1290 EFFECT OF FOOD DEPRIVATION ON SLEEP IN THE RAT. Alexander A. Borbély. Institute of Pharmacology, Univ. Zürich, CH-8006 Zürich, Switzerland.

Continuous telemetric EEG-recordings served to determine the vigilance states of the rat during 2 control days, 80 hr of food deprivation, and 64 hr following restitution of food. The recordings were supplemented by measurements of food intake, water intake and motor activity. The following sleep parameters were not significantly changed throughout the experiment: the daily amount of the vigilance states; the light-dark distribution of sleep and waking; and the 10-min paradoxical sleep (PS) cycle. During food deprivation, PS was depressed in the dark phase of the diurnal cycle, and increased in the light phase. The duration of sleep episodes was the parameter that was most affected by food deprivation. Episodes of slow wave sleep (SWS) and PS were shortened only in the dark phase of the deprivation days, whereas total sleep episodes were progressively decreased in both diurnal phases. After restitution of food, the episodes of SWS and total sleep were immediately lengthened and tended to exceed the control level. The duration of feeding episodes and meal size were also significantly increased in comparison to the control values, whereas feeding frequency was decreased. The changes of nocturnal motor activity episodes paralleled those of the sleep episodes. It is proposed that the adjustment of the length of behavioral episodes may constitute an important adaptive mechanism. (Supported by the Swiss National Science Foundation, grant 3.2120.73).

1291 TIME COURSE OF "RESPONSE-REVERSAL" PHENOMENON: THE EFFECTS OF RETICULAR STIMULATION ON REFLEX ACTIVITY DURING SLEEP AND WAKEFULNESS. Michael H. Chase, Nancy K. Wills-Lewis*, and Margaret I. Babb*. Depts. Physiol. and Anat., Sch. Med., UCLA, Los Angeles, CA. 90024.

We have recently described a phenomenon in the central nervous system wherein stimulation of a single reticular locus yields different somatic responses which are determined entirely by the state of the animal. Specifically, we reported that reticular stimulation in the region of the ponto-mesencephalic junction led to facilitation of the masseteric reflex while the animal was awake or in quiet sleep and profound inhibition of the reflex during active sleep. We have called this state-dependent phenomenon "response-reversal".

As a first step in the identification of synaptic connections responsible for "response-reversal", we examined the time course of the effect in 8 chronically implanted freely-moving cats. Short pulse train stimulation of the reticular formation of the ponto-mesencephalic junction was paired with a masseteric reflex-inducing pulse to the mesencephalic Vth nucleus at conditioning-test intervals of 5 to 100 msec. During wakefulness and quiet sleep, strong reflex facilitation (>50% of baseline) was observed at intervals of 15 to 25 msec. During active sleep, however, strong inhibition (<50% of baseline) was found at the same intervals (15 to 25 msec). The period of facilitation during wakefulness and quiet sleep coincided with the period of inhibition during active sleep.

The above findings suggest that the facilitory and inhibitory components of the "response-reversal" phenomenon may be mediated by pathways with comparable synaptic delays. Since the effects occurred at comparable conditioning-test latencies, similar synaptic mechanisms may be responsible.

1292 ELECTROCONVULSIVE SHOCK: PROLONGED EFFECTS ON SLEEP AND ELECTROCORTICOGRAPHIC ACTIVITY IN THE RAT. A.M.DeLuca*, S.L.Chorover and R.T.Pivik, Dept. of Psychology, Massachusetts Institute of Technology, Cambridge, Mass. 02139 and Dept. of Psychiatry, Ottawa General Hospital, Ottawa, Ontario.

A single electroconvulsive shock can induce prolonged effects on slow-wave sleep (SWS), and cortical steady potentials (SP) in rats. The EEG, SP, EMG, and sleep patterns were recorded continuously for 48-144 hours prior to the ECS and for 7-30 days following treatment. The ECS (35-50mA, .2sec, 60Hz) was delivered through bilateral skull screws over the anterior cortex. The recording was done from the home cage in which each animal was maintained on a 12 hour light - 12 hour dark cycle.

SP shifts related to the transition from quiet wakefulness to SWS and from SWS to PS were cortical negative, 100-200μV and 200-500μV respectively. Upon awakening, there was a positive shift to the waking SP levels. All ECS treatments elicited grand mal electrical and behavioral seizures. ECS-induced shifts were negative, 4.5-8mV. The initial negativity was maintained for 10sec-3min. Behavioral recovery to a standing position was achieved in 3-16min. In 3 of 4 animals a substantial increase in total sleep time (TST) persisted for 3 days following ECS. The increased TST was accompanied by an absolute reduction in paradoxical sleep, and was largely concentrated in the dark portion of the light-dark cycle. In marked contrast to the SP stability obtained prior to ECS, the SP of every animal underwent major, irregular and unpredictable shifts. These began as early as 10min. after ECS and lasted 1-3 days. Their magnitude was greater and their duration longer than those seen during the normal sleep cycle. They occurred both in the presence and the absence of EEG spike activity.

These changes in sleep patterns and SP which persists for up to 72 hrs. after ECS may reflect changes in brain metabolic activity.

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1293 SLEEP STATE PERIODICITY DEVELOPMENT IN NORMAL INFANTS AND INFANTS AT RISK FOR THE SUDDEN INFANT DEATH SYNDROME. R. M. Harper*, T. Hoppenbrouwers*, D. Bannett*, D. J. McGinty, M. B. Sterman, and J. Hodgman*. Sepulveda VAH and Dept. of Anatomy, UCLA, and County USC Medical Center.

Ten normal infants and ten infants at risk for sudden infant death were instrumented with EEG, EOG, respiratory, somatic activity and ECG electrodes and recorded for 12-hr periods at 1 week and 1, 2, 3, 4, and 6 mos. of age. Successive minutes of each 12-hr recording were classified as QS, REM, waking or transition states by trained observers. These state data were then transformed into binary sequences of state presence or absence and subjected to Fourier transform spectral analysis. Analysis of variance procedures were used to assess developmental trends and risk effects. In both normal and risk infants, QS and REM spectra showed shifts during this period of development from distributions characterized by wide peaks to ones dominated by narrow peaks centered at 1 cycle/hr. There was no significant difference between the peak frequency of QS and REM spectra at any age. REM state periodicities at frequencies below .2 cycles/hr developed differently in normal and risk infants. Power in the awake spectra was distributed differently in the two groups, with risk infants showing relatively more power at frequencies below .6 cycle/hr and less at those above. Disturbance of sleep state periodicity may play a role in sudden infant death, possibly by alteration of cardiac and respiratory functions associated with changes in state.

1294 CHANGES IN SLEEP PATTERNS ASSOCIATED WITH ACUTE WITHDRAWAL FROM PURE HEROIN. Richard C. Howe and Frederick W. Hegge*. Dept. Phys. & Bioeng., Eastern Virginia Med. Sch., Norfolk, VA. 23501 and Dept. Psychophys., Walter Reed Army Inst. Res., Washington, D.C. 20012.

The purpose of this study was to analyze EEG records obtained from heroin dependent individuals during acute withdrawal. The study was conducted on a hospital research ward. The drug dependent individuals consumed approximately 1025 mgm of 92-98% pure heroin daily for an average of 4.2 months (before entering study). All data were collected on a 24-hour per day basis for 5-7 days using biotelemetry techniques. The EEG records were analyzed for various awake and sleep states according to standard techniques. From a total of 25 subjects, 5 control subjects and 4 drug dependent patients have been analyzed to date. All scored EEG data were transfered to computer files for subsequent analysis.

The preliminary results indicate that the control subjects exhibited fairly standard sleep patterns in that they showed the typical "first night effect" followed by a stabilization of sleep on consecutive nights and that the percentages of the sleep-waking states were within normal values. The patients undergoing heroin withdrawal displayed a disruption in the sleep pattern characterized by an increase in the awake states, a decrease in some of the slow wave sleep states and a greater suppression of the REM sleep state. In addition, the control subjects tended to sleep during a continuous block of time, whereas the heroin patients during withdrawal showed attempts to sleep throughout the day. Further analysis of the EEG data from the remaining heroin dependent patients is necessary to more adequately describe these alterations in the sleep-waking patterns.

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1295 PGO WAVES: PHASE LOCKED FIRING BY PONTINE RETICULAR NEURONS. Robert W. McCarley and J. Allan Hobson. Dept. of Psych., Harvard Medical School, Boston, Mass. 02115

Correlational criteria for identifying generator cells for the ponto-geniculate-occipital (PGO) waves of desynchronized sleep (D) include long lead times (phasic latency) and the degree of phase-locking. In an earlier report we described the cross-correlation between the activity of units recorded in the FTG and the desynchronized sleep PGO waves recorded at occipital cortex. Although the statistical association was clear, the FTG discharge trains failed to show the degree of consistent, tight phase locking that one might expect of generator neurons. We now report the discovery of pontine reticular cell activity with equally long lead times with respect to isolated PGOs but with a more impressively consistent tight phase relationship to the PGO waves. Cells were recorded over naturally occurring sleep-waking cycles in cats prepared for chronic micro-electrode, EEG (parietal and left lateral geniculate), EMG and EOG recording.

We judged 11 cells or 13% to have strong phase locking between unit discharge and isolated LGN PGO waves. Ten of these 11 cells were between 1.5 and 2.3 mm lateral to the midline, a zone encompassing previously reported "hot spots" for selectivity, intensity of clustered discharge, and eye movement correlated discharge. One was at a midline location in TRC. On a rostro-caudal dimension 4/11 sites were in the FTG, and 6/11 sites were in the paramedian reticular formation just rostral to the FTG, in the FTC. Two physiological features characterized these units. 1) long lead time of clustered unit discharge vis-a-vis the isolated LGN PGOs; lead times may exceed 800 msec. 2) Tight coupling between the discharges of the unit and the occurrence of PGO waves, although it is still not completely one-to-one. More rostral units appear to have shorter lead time with tighter phase locking than those more caudal.

1296 BREATHING DURING SLEEP AND WAKEFULNESS IN THE CAT. John Orem*, Allan Netick*, and W. C. Dement. Stanford Univ., Sch. Med. and Cal. State University, Hayward.

Seven adult cats were equipped with standard electrodes and skull bolts for chronic recordings with head-restraint. Respiration was monitored through a tracheal fistula with a pneumotachograph. Since airflow bypassed the upper airway, the measurements were of the output of the respiratory pump. Each cat was studied for a minimum of 8 hours on each of 2 recording days. They were sleep deprived prior to recordings, and after experiments, episodes of Wakefulness (W), nonrapid eye movement (NREM) and rapid eye movement (REM) sleep were played back, and airflow parameters were digitized for computer analysis.

The combined means and the standard errors of the mean for frequency of breathing (F), peak inspiratory airflow rate (PF), tidal volume (Vt) and minute-volume ($\dot{V}e$) were as follows. (1) F was lower in NREM (25.5±.35/min.) than in W (37.5±1.5) and REM (41.8±1.9). (2) Changes in F across states involved changes in both the lengths of inspiration (Ti) and expiration (Te), although in progressing from W to NREM and REM, the ratio ti/Ti+Te progressively increased. (3) PF progressively decreased from W (4.324±.09 liters/min) to NREM (3.03±.04) and REM (2.572±.05). PF was positively correlated with Vt (+.5 to +.9). (4) Because of a greater Ti, Vt was larger in NREM (35.1±.43 ml) than in W (33.2±.73). A short Ti and a minimal PF produced a small Vt in REM (24.4±.63). (5) Within each state Vt and F were negatively correlated (-.3 to -.7). (6) $\dot{V}e$ decreased in sleep (W=1081±25 ml; NREM=883±9.9; REM=806±19). (7) Ss were studied with dead spaces added to the pneumotach. Although ventilation parameters increased, the effects of state on breathing, as determined without added dead space, were confirmed.

1297 SIMILAR EFFECTS OF TRYPTOPHAN AND SLEEP ON CISTERNAL CEREBROSPINAL FLUID 5-HYDROXYINDOLEACETIC AND HOMOVANILLIC ACIDS IN CATS. M. Radulovacki, R. L. Buckingham, E. H. Chen* and R. Kovacevic. Departments of Pharmacology and Biometry Program, Univ. Ill. Med. Ctr., Chicago, Ill. 60612.

We administered L-tryptophan to cats to find out whether chemical changes in cerebrospinal fluid (CSF) produced by tryptophan parallel those occurring during natural slow-wave sleep (SWS). In order to obtain CSF, we implanted cats with a cannula in the cisterna magna. Control experiments were conducted in animals which were kept awake for 1 hour, after which time 1 ml of CSF was taken. After maintaining one additional hour of wakefulness a placebo capsule was administered orally and CSF (1 ml) was withdrawn 1 hour after placebo administration. The same procedure was used when a capsule containing 10 mg of L-tryptophan was administered. 5-Hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), the end products of 5-hydroxytryptamine and dopamine metabolism, respectively, were determined by the method of Korf and Valkenburgh-Sikkema. Data for CSF 5-HIAA and HVA during SWS were obtained from our previous study. Thus, the data were obtained for four behavioral states, namely, during SWS, wakefulness, after placebo, and tryptophan.

Our results show that the mean levels of HVA in the four behavioral states are not significantly different ($P = 0.372$, using one-way analysis of variance) and that the mean levels of 5-HIAA in the four behavioral states are significantly different ($P < 0.0005$). The analysis further indicates, at 5% level of significance, the existence of three subsets among the four behavioral states with respect to mean levels of 5-HIAA. The first subset contains only wakefulness (mean = 82.7), the second contains only placebo (101.4) and the third contains both tryptophan (120.5) and SWS (124.5). These findings suggest similar effects of tryptophan and SWS on CSF 5-HIAA and HVA in cats.

1298 TREATMENT OF THE SLEEP APNEA SYNDROME WITH THEOPHYLLINE. M. Shkurovich*, J.C. Ugartechea*, G. Valencia*, E. Jurado*, J. Vega*, and R.R. Drucker-Colín. Hosp. del Niño, IMAN and Inst. Biol. Univ. Nac. Auton. Méx., México, D.F.

Recently it has been suggested that the sleep apnea syndrome (SAS) is directly related to the sudden infant death syndrome (SIDS). SAS is related to immaturity and depression of the CNS mechanisms controlling cardio-respiratory functions. This study reports the effects of theophylline, a CNS stimulant on SAS. Six high risk patients (2 weeks to 5 months of age) out of 25 subjects with SAS were selected for theophylline treatment. Polygraphic studies were performed during morning naps for periods of two to four hours and evaluated by conventional EEG methods, before and after oral administration of 1 to 4 mg/kg of theophylline. Each subject was studied between 3 and 14 times. In the control studies, the sleep apneas occurred at a frequency range of 22 to 246 times with a duration of 2 to 140 secs. The range percent time of SWS apneas was 1.3 to 20.7% of the SWS time and 1.23 to 10.50% of REM time. After the administration of theophylline SWS apneas were reduced to a range of 0 to 3 secs with a range time of 0 to 1.97% of total SWS time and REM apneas range frequency was reduced to 3 to 67 secs with a range time of .003 to 3.6% of REM time. All patients showed an increase in REM. Our data suggests that there may be two types of SAS, one during SWS and highly responsive to theophylline treatment and the other occurring during REM but more resistent to the effect of this drug. It is suggested that theophylline is effective in the treatment of SAS and can prevent SIDS.

1299 WAKING ACTIVITY IN PONTINE FTG NEURONS. Jerome M. Siegel and Dennis J. McGinty. Veterans Administration Hospital, Sepulveda, California 91343 and Depts. of Anatomy and Psychology, UCLA, Los Angeles, California

We have previously reported the presence of waking activity in those neurons in the pontine gigantocellular field (FTG) which show a marked increase in rate in rapid eye movement sleep (REMS). The present study attempts to further define the stimuli responsible for the excitation of the FTG neurons in waking. Recording from unrestrained cats, we found that all FTG neurons responded to sensory stimuli applied during waking. Of the 20 units subjected to systematic behavioral testing, 13 responded to vestibular stimulation, 7 to somatic stimulation, and 5 to auditory stimulation. Six responded to more than one sensory modality. Most vestibular units showed increased firing with head movement in one direction and decreased firing with movement in the opposite direction. Units responding to somatic stimulation had receptive fields ranging in size from small ipsilateral fields covering a 2 cm^2 area, to large bilateral fields encompassing the entire body. Natural pressure and stroking was accompanied by rapid discharge lasting for periods of 10 seconds or longer. Single 0.5 msec shock stimuli applied to the receptive fields produced a consistent but brief response beginning at a latency of from 15 to 28 msec in different units. Some units which responded briskly to natural somatic stimuli showed no response to shock stimuli. Cells responding to auditory stimuli fired at a 13-18 msec latency. Other workers (Hobson et al., 1974)using restrained cats have emphasized the selectivity of discharge in these neurons for REMS. However, we find that discharge rates in waking will increase significantly in the presence of appropriate stimuli. Thus, the apparent selectivity of discharge in these neurons for REMS will vary as a function of the presence of stimulation during the waking period selected for comparison.

1300 SELECTIVE FIRING OF RAT PONTINE GIGANTOCELLULAR NEURONS DURING MOVEMENT AND REM SLEEP. Robert P. Vertes* (SPON: K. L. Casey) Department of Physiology, University of Michigan, Medical School, Ann Arbor, MI 48109.

In the restrained cat, Hobson et al. (J. Neurophysiol. 37:497, 1974) have found that neurons of the pontine gigantocellular field (PGF) fire at very slow rates during waking and slow wave sleep, and increase their discharge as much as 100 times during selective segments of REM sleep. Hobson (Advances in Sleep Research, E. Weitzman [Ed.] 1974, p. 217) has proposed that PGF cells form the center for the control of REM sleep.

In the present study, 18 PGF neurons in 15 freely-moving rats were recorded from during four states: waking (W), waking with movement (W-M), slow-wave sleep (SWS), and rapid eye movement sleep (REM). Fifteen of these 18 PGF neurons fired significantly more in REM than in either W or SWS as described by Hobson et al. (above ref.) for cat PGF cells. However, each of these 15 neurons also showed a significant increase in firing rate during waking with movement; in fact the mean firing rate for these cells was slightly greater in W-M than during REM. These results indicate that the high frequency discharge of PGF cells is not uniquely associated with the onset or control of REM sleep. It is possible that species and sampling differences have allowed us to record from PGF neurons with properties markedly different from those in the cat. The location and sleep associated firing rates of our PGF neurons, however, are quite comparable to those of Hobson et al.

The selective firing of PGF neurons during movement and REM sleep could be related to the somatic motor events common to both states: alternatively PGF activity could be associated with the hippocampal theta rhythm which is also selectively present in the rat during movement and REM sleep. In any case, it is apparent that the function of these PGF cells extends beyond the REM sleep state. (Supported by NIH Fellowship Grant 1 F32 NS05300-01 and by NIH Grant 1 R01 NS12015-01).

Somatosensory Systems

1301 REBOUND EXCITATION AND THALAMIC RHYTHMIC ACTIVITY. T. Bando, A. Zambelli* and W.A. Spencer*, Div. Neurobiol., Dept. Physiol. Coll. of Phys. & Surg., Columbia U., New York, N.Y. 10032.

The role of recurrent inhibitory phasing in the genesis of thalamic rhythmic activity has been emphasized (Andersen and Eccles, 1962). However, there has been disagreement among investigators on the mechanism generating rebound bursts of discharges which follow the triggering stimulus evoked IPSPs. In studies of the ventral postero-lateral nucleus (VPL) of the thalamus, Andersen et al. (1964) postulated that, during the late decaying phase of peripherally or cortically evoked IPSPs, thalamocortical relay cells were in a hyperexcitable state due to postanodal exaltation. This hypothesis was challenged by Maekawa and Purpura (1967) who found rebound discharges after induced membrane hyperpolarization only in injured cells in VPL. However, the sequential rhythmic burst activity triggered by Andersen et al. was not well developed in the records of Maekawa and Purpura. Therefore further work is necessary to clarify the mechanism of rebound bursts of discharges in VPL relay neurons.

In the present study membrane potential changes of VPL neurons evoked by stimulation of either a cutaneous nerve or the postcruciate cortex were analyzed. To maintain the rhythmic activity of VPL neurons, special care was taken to keep cats at moderately light anesthetic levels with pentobarbital sodium. Thalamocortical relay cells were identified antidromically. A single cutaneous or cortical stimulus evoked an initial EPSP followed by the IPSP. On the late declining phase of the IPSP, clustered rebound depolarizations were often seen to generate a burst of spikes. These depolarizations were followed by several similar cycles.

Rebound depolarizations were not due to relay cell membrane postanodal exaltation: 1) they were found even when the size of IPSPs was reduced to a few millivolts by decreasing the stimulus intensity, and 2) similar depolarizations often appeared without being preceded by hyperpolarizations. Moreover, after inversion of IPSPs by injection of chloride ions through the microelectrode, "rebound" responses remained as depolarizing potentials. Based on this evidence we propose that the rebound bursts are caused, in large part, by clusters of late EPSPs.

(Supported by 2 RO1 NS 12744 NEUA)

1302 THE CONSEQUENCE OF NEONATAL VIBRISSAE REMOVAL ON TRIGEMINAL PATHWAYS IN THE RAT. II. DEVELOPMENT OF ANOMALOUS BRAINSTEM PATHWAYS (PRIMARY AND SECONDARY AFFERENTS). G. Belford* and H.P. Killackey (SPON: M. Snyder). Department of Psychobiology, University of California, Irvine, CA 92717.

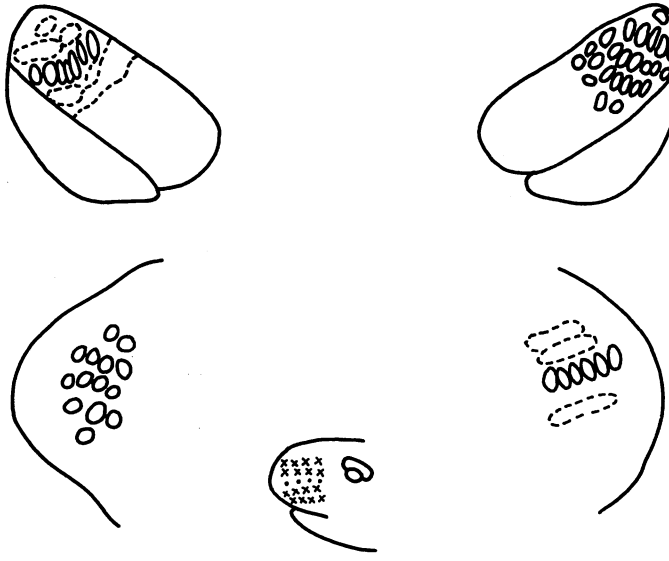
We have previously described segmentation in the principal trigeminal nucleus and the ventrobasal complex (Anat. Rec., 1976, 184, 446). This segmentation occurs in the portions of these nuclei that have been shown to be related to the vibrissae by electrophysiology. Further, the pattern of segmentation is strongly reminiscent of the barrel field in somatosensory cortex. For these reasons we postulated that this segmentation is related to the vibrissae in a one-to-one fashion. We tested this hypothesis by removing vibrissae and observing the consequent organization in these two nuclei.

On the day of birth littermate rats had vibrissae rows A,B,D and E on the right side of the face electrocauterized and removed leaving row C intact. These animals were sacrificed on postnatal days 6 and 7. Brains were sectioned in the coronal plane and tissue sections were processed for SDH histochemistry.

Anomalous segmentation is observed in both the trigeminal nucleus and the ventrobasal complex. In the trigeminal nucleus the middle row of segmentation (corresponding to the intact row C) stains with a normal density, whereas the adjacent rows are less densely stained and less discretely segmented than normal counterparts.

Within the ventrobasal complex the changes are more dramatic. Segmentation corresponding to row C is enlarged compared with that in the contralateral control nucleus. The areas corresponding to rows A,B,D and E do not show normal segmentation; instead, a distinct band is present corresponding to each row of removed vibrissae with some tendency towards segmentation with a band.

These results indicate that the segmentation seen in the trigeminal nucleus and ventrobasal complex are definitely related to the vibrissae and that peripheral intervention can induce anomalous organization in multiple central structures. Comparison of these results with the anomalous organization seen in cortex indicates developmental similarities in both cortical and non-cortical structures. SUPPORTED BY NSF GRANT #GB 41294.



1303 PROJECTIONS TO THE INTRALAMINAR GROUP COMPLEX OF NUCLEI IN THE CAT THALAMUS. K. J. Berkley. Dept. Psychol., Fl. St. Univ., Tallahassee, FL. 32306.

Cells of origin of spinothalamic tract fibers in the cat possess a wide variety of response properties. These fibers, located in the ventral and lateral portions of the spinal cord project to at least three separate zones within the diencephalon--the border between n. ventralis lateralis (VL) and n. ventralis posterolateralis (VPL), the n. centralis lateralis (CL) of the intralaminar group complex of nuclei, and the medial division of the posterior group complex of nuclei (POm).

In order to better understand this dispersive termination pattern, the afferent connections to each of these three thalamic recipient zones are presently being studied using a differential labeling strategy. By using two different methods to label two different pathways in the same cat (autoradiographic and degeneration methods), this strategy permits direct observation of the regions where preterminals and terminals from different afferent sources may overlap. It is reasoned that this type of anatomical analysis might reveal differing patterns of convergent connectivity within each of the three zones that could in turn be functionally related to the observed differences in spinothalamic cell properties.

In an earlier study reported last year at these meetings, and in a paper to be presented this year (Mash, et al.), it has been found that the VL-VPL border region that receives spinothalamic input also receives input from the cerebellum, the n. Z, the lateral cervical nucleus and the ventral portions of the cuneate nucleus. These results suggest that the VL-VPL border region as well as the spinothalamic tract and lateral cervical nucleus fibers projecting to it may have some function in proprioception.

In the present experiment, the connections of the intralaminar group complex of nuclei have been similarly investigated. It has been found that, similar to the VL-VPL border zone, the zone within CL to which fibers in the spinal cord project also receives input from the deep cerebellar nuclei. On the other hand, this region does not appear to receive input from the n. Z, the lateral cervical nucleus, or the n. cuneatus. Furthermore, although there may be some overlap, preliminary evidence suggests that the trigeminal n. (pars caudalis), the somatosensory areas 1 and 2 and the somatomotor area 1 of the cerebral cortex all appear to send projections predominantly to other portions of the intralaminar complex.

These results show that, despite some similarities, the pattern of connectivity of the VL-VPL border region differs from that of the intralaminar region. When considered together with the results of other investigators, the connections of the former suggest a role in proprioception, whereas the connections of the latter tentatively suggest a role in muscle pain.

(Supported by PHS grants 1K04-NS00118 and 5R01-NS11892 from the National Institutes of Health.)

1304 DESCENDING PROJECTIONS FROM MEDULLARY SOMATOSENSORY RELAY NUCLEI. H. Burton and A.D. Loewy, Depts. Anat. & Neurobiol. and Physiol., Wash. Univ. Sch. Med., St. Louis, Mo. 63110.

The dorsal column nuclei (DCN) are generally regarded as part of an ascending somatosensory relay system although Dart (J. Physiol., 219:29P, 1971) reported that some DCN neurons could be antidromically activated by electrical stimulation of the dorsolateral funiculus at the C6 level. Retrograde labeling with horseradish peroxidase (HRP) was combined with various spinal cord lesions to identify the cells and pathway of this descending system. In cats and monkeys (M. fascicularis) 2 injections (~0.2 µl) of 25% HRP were made at each of 10-15 sites that were positioned at 2 mm intervals along the lower cervical and/or upper thoracic segments. Lesions involving various parts of the dorsal, lateral, or ventral columns were made at the C3 level, and 2 cm rostral, and prior to the injections in order to demonstrate, by negative evidence, the general location of the descending axons.

Labeled cells are present ipsilateral to the injections in ventral and rostral portions of the gracile and cuneate nuclei and are heavily concentrated in between these two nuclei. Large stellate cells lying on the periphery of the cell islands are labeled together with small fusiform cells which are concentrated along the ventral border of the DCN. The stellate cells correspond morphologically to cells shown by Cajal to send their axons into the dorsal columns. Complete lesions of the dorsal columns (DC), which spared the dorsolateral funiculus (DLF), causes almost total loss of labeling in the DCN. Lesions involving only the medial half of the DC or the DLF have little or no effect on the number and distribution of retrograde labeling.

A small number of labeled neurons are present in the ipsilateral magnocellular and marginal divisions of the spinal caudalis nucleus of V (Sp.V). Large, stellate cells were identified in the magnocellular division of SpV; large, fusiform cells were seen in the marginal division of SpV. Lesions involving the medial aspect of the DLF prevents retrograde labeling of these cells.

The terminal distribution and course of the descending DCN fibers was demonstrated with autoradiographic anterograde labeling. Microinjections of .015-.035 µl were made with micropipettes containing 50 µCi/ul ^{3}H-proline. Placement of the injections was guided by recording with the injection electrode. The sequence of tactile receptive fields was sought which would indicate that the electrode was in the portion of the somatotopic map corresponding to the region containing the greatest number of neurons with descending axons.

After 5-6 days survival, the autoradiographs of the spinal cord show that silver grains cluster over bundles of axons within the DC. At upper cervical levels these bundles are prominent along the lateral and deep aspects of the ipsilateral DC. Close to the terminal fields the labeled bundles curve over the dorsal and dorsolateral portion of the dorsal horn. A pattern of dispersed labeling is present over the lateral cervical nucleus and Rexed's laminae I, IV and V. The greatest concentration of this terminal pattern of grains is distributed over the medial half of lamina V in the cervical spinal cord.

The functional significance of these descending connections is unknown although it is tempting to speculate that they may modulate activity in other ascending somatosensory tracts. (Supported by USPHS Grants NS 09809, NS 12751 and RR 05389)

1305 RESPONSES RECORDED FROM HUMAN SCALP EVOKED BY SKIN WARMING.
Allen B. Chatt and Dan R. Kenshalo. Dept. Psychology, Florida State University, Tallahassee, Florida 32306

This study demonstrates that thermal evoked responses to warming the glabrous palmar skin can be recorded from a contralateral parietal scalp site that approximates the hand projection area of sensorimotor cortex when the adapting temperature (AT) is maintained at 35° C rather than at 30° C as was the case in an earlier study. Though the response was recorded maximally from the contralateral scalp of 4 of 5 Ss demonstrating a response, in 2 of the Ss a smaller and later occurring response was also found at the ipsilaterally identical site. Negative results from the stimulus control sessions affirm that the response was due to skin temperature change and not to some artifact of stimulation. Peak latencies ranged from 280 msec to 356 msec to stimulus intensities of 8°C presented at a rate of 1° C/sec. These latencies were nearly twice as long as thermal evoked responses evoked by cooling the thenar eminence at similar rates. This warm evoked response would appear to have its origin in the specifically sensitive warm fiber afferents since the success of the 35°C AT in demonstrating a response coincides with the greater dynamic sensitivity of these afferents at this higher AT. Multi-modal nociceptive fibers are known to respond to skin warming but they do not function within the temperature range used in this study. The presence of warm, cool and tactile evoked responses to palmar stimulation at virtually identical scalp sites in man has some interesting implications concerning the functional organization of sensorimotor cortex in all mammals. (Supported by USPHS Grant NS-02992)

1306 SUBCORTICAL PROJECTIONS FROM CYTOARCHITECTONIC FIELDS OF THE SOMATIC SENSORY CORTEX IN MONKEY. J.D. Coulter and E.G. Jones, Marine Biomedical Institute, Depts. Physiol. and Biophys., and Psychiat., Univ. of Texas Medical Branch,, Galveston, Texas 77550 and Dept. Anat. and Neurobiol., Washington Univ. School of Medicine, St. Louis, Missouri 63110.

The distribution of the subcortical projections from areas 3, 1 and 2 of the postcentral gyrus was examined by the autoradiographic method in 15 cynomolgus monkeys (M. fascicularis). A row of 5-10 injections were placed in the cortical representation of the hand in cytoarchitectonic area 3b, 1 or 2 in different animals. For comparison similar injections were made in other animals in areas 3a, 4 or 5. Injections were made using air pressure through a glass micropipette (dia. 20-50 µm) with each consisting of 3.0-4.5 µC of [^{3}H] proline and leucine in 0.06-0.09 µl of normal saline. After survival times of 5-14 days, the animals were killed and the brains and spinal cords prepared for autoradiographic tracing of connections. Injections confined to areas 3b, 1 or 2 in all cases led to terminal labeling in the putamen, thalamus and pontine nuclei, ipsilateral to the injected hemisphere, and in the cuneate nucleus and cervical spinal cord contralaterally. Multiple burst-like patches of labeling were seen in the putamen, while in the thalamus there was labeling throughout the dorsal-ventral extent of the forelimb sector of the ventrobasal complex and in parts of the reticular and central lateral nuclei. In the dorsal column nuclei, labeling was confined to the deepest part of the cuneate nucleus, and in the pons, multiple, widely separated bands of labeling extended through the full rostral-caudal extent of the pontine nuclei. In the spinal cord, labeling was restricted to a short rostral-caudal portion of the cervical enlargement where it was centered over the dorsal-medial spinal grey matter. While few differences were noted in the distribution of labeling to other subcortical structures, injections in the different cortical fields did result in differential patterns of labeling in the spinal cord. After injections of area 1, labeling was largely confined to the medial half of the dorsal horn, corresponding to Rexed's (1954) lamina IV of the cat. Injections of area 3b led to labeling extending more laterally and dorsally, encroaching on lamina III. By contrast, injections of area 2 resulted in labeling of medial lamina IV, with extension deep into medial lamina V and into the dorsal part of lamina VI. The results indicate that each of the cytoarchitectonic fields 3b, 1 and 2, of the primate somatic sensory cortex have extensive subcortical projections to the thalamus, brainstem, and spinal cord. With respect to the spinal cord, there further appears to be a differential termination of the projections from the different cortical areas to different parts of the dorsal horn. In view of the known segregation of response properties of neurons in the different cytoarchitectonic fields of the somatic sensory cortex and the differential distribution of their efferents in the dorsal horn, the possibility exists that these cortical areas selectively influence different classes of spinal cord sensory activities. (Supported by NIH grants NS 12481 and NS 10526.)

1307 CODING OF INCREMENTAL INCREASES IN SKIN TEMPERATURE BY WARM FIBERS IN THE MONKEY. Ian Darian-Smith, Kenneth O. Johnson, Carole LaMotte, Y. Shigenaga* and C.M. Vun*. Dept. Physiol. Univ. Melbourne, Victoria, Australia, and Dept. Physiol., Johns Hopkins Univ. Sch. Med. Baltimore Md.

Previous investigation (LaMotte et al., Soc. Neuroscience 2nd Meeting 1972) showed that the warm fiber population innervating the macaque monkey's palmar skin is a functionally homogeneous group of C fibers. It was also shown that intensity functions relating a single warm fiber's response to a near-rectangular warming pulse at amplitudes of 0-10C (T-step) are monotonic and that their form is determined by (a) the response measure, (b) the baseline temperature (T-base) and (c) the pattern of stimulation in the 30 secs preceding the test stimulus.

When the determining stimulus parameters outlined above are fixed, and when the response measure for a single fiber is the cumulative impulse count over a defined interval following the onset of the warming pulse, then the minimum incremental increase in the intensity of a warming pulse which can be resolved with a probability of 0.75 by those responses is defined by the relationship

$$DL' = \sigma_R / \left(\frac{dR}{DI}\right)$$

where DL' = the increment in a pair of cooling pulses differentiated with a probability of 0.75

σ_R = standard deviation of warm fiber response to stimulus of fixed amplitude (at which resolution is measured)

$\frac{dR}{dI}$ = mean sensitivity of warm fiber to incremental changes in stimulus intensity (Darian-Smith et al., Johnson et al., J. Neurophysiol. 36: 325-370, 1973).

If the responses of n warm fibers synchronously responding to the successive stimuli are averaged the stimulus increment which can be defined (with a probability of 0.75) becomes

$$\frac{1}{\sqrt{n}} \cdot \sigma_R / \left(\frac{dR}{dI}\right)$$

when each of these fibers responds independently to the stimuli.

The value of DL' was determined for single warm fibers over a wide range of T-base and T-step values for trains of warming pulses 4 seconds induration, recurring every 10 seconds. In these determinations the response measure was the cumulative impulse count over successively longer periods following the onset of the stimulus. These data were also used to estimate (a) the resolution of incremental increases in the intensity of the warming pulse achieved when the response measure was the averaged response of fiber samples of known size, and (b) the effect on the DL' of dependent variability of response within these fiber populations.

These estimates of DL' were matched with the trained human subject's ability to assess the incremental intensity difference between pairs of warming pulses. The present investigation extended earlier psychophysical observations (LaMotte, C., Ph.D. thesis, Johns Hopkins Univ, 1972) in that the effect of stimulus area (thereby increasing n) was also examined. From these correlative studies the following conclusions were made: The warm fiber population signals sufficient information to the CNS about the intensity of a warming pulse to account for the human subject's ability to differentiate stimuli if, and only if,

(a) the input from the whole warm fiber population engaged by the stimulus is used by the CNS
(b) the dependent variability of the responses of synchronously excited warm fibers is small
(c) that transmission of this information in the sensory pathways and the decision-making process involve little or no loss of stimulus information.

1308 THE CENTRAL REFLECTION OF PRIMARY AFFERENT RESPONSE PROPERTIES ASSOCIATED WITH THERMAL STIMULATION OF THE SKIN.
Roland Duclaux*, Allen B. Chatt and Dan R. Kenshalo.
(Spon: Hilton Stowell) Dept. Psychology, Florida State University, Tallahassee, Florida 32306.

In a recent study, we reported the presence of a thermal evoked response at a 29°C adapting temperature (AT) and its absence at a 40°C AT when the glabrous palmar surface in man was cooled. From this, we concluded that a specifically sensitive cold fiber population exists in man that is reflected centrally by this cool evoked response from scalp. In reconciling this data with afferent cold fiber data obtained from Rhesus median and ulnar nerves however, it became clear that the peak dynamic frequency of these fibers were virtually identical at both these ATs. Clearly, then, this afferent fiber response parameter cannot account for the differential response reported in man.

Three response parameters have now been identified which appear to be more adequate correlates of the central evoked response: onset latency, peak latency and instantaneous frequency which is the rate of response rise between response onset and peak. At 29°C AT where thermal evoked responses were readily recorded, the instantaneous frequency of the afferent fiber population was nearly twice as great as that at the 40°C AT where central evoked responses were not recorded. In addition, the onset and peak latencies at the lower AT were from 2 to 3 times shorter than at the higher AT. At the 29°C AT, increases in stimulus rate were reflected centrally by a decrease in the peak latency of the thermal evoked response. Cold fiber afferents respond in a similar manner with their onset and peak latencies decreasing with faster stimulus rates. SAI mechanoreceptors have longer peak latencies and smaller instantaneous frequencies to a cooling pulse at all ATs between 20-40°C than do cold fibers at 40°C. If the longer latencies of response onset and peak frequency and smaller instantaneous frequency account for the failure of cold fibers to elicit an evoked response at the 40°C AT, then SAI mechanoreceptors could not be expected to elicit an evoked response to cooling.

Clearly then, these response parameters possess the temporal characteristics necessary for the transmission of a highly synchronized burst of neural activity to the CNS. Instantaneous frequency would appear to be the parameter most closely related to this synchrony, with onset latency and peak latency being reflected in the peak latency of the central phenomenon. Peak dynamic frequency does not appear to be related critically to the central occurrence of an evoked response at least in the thermosensory system.
(Supported by USPHS Grant NS-02992 and NSF Grant GB-30610.)

1309 HISTOLOGICAL DEFINITION OF THE THIRD MYSTACIAL VIBRISSA REPRESENTATION IN CATS. R.W. Dykes and E.G. Jones. Depts. of Physiology and Biophysics, Dalhousie Univ., Halifax, N.S., Canada, and Dept. of Anatomy, Washington Univ., St. Louis, Mo.

That the somatosensory representation was triplicate, at least for some body parts was discovered by Marshall, Woolsey and Bard (J. Neurophysiol., 4:1, 1941), but it was not studied seriously until Darian-Smith and colleagues (Ann. Rev. Physiol., 31:417, 1969) studied the cortical projection of the face of cats. More recent data have provided conflicting evidence on the number of somatotopically organized body representations in the cortex. In order to further characterize these projections in cat, detailed evoked potential maps of individual vibrissae were obtained and the sites were marked for later histological study. The results of the evoked potential maps show that the sites for the projections of the mystacial vibrissae were highly variable and support the argument that histological controls are necessary to confirm the proper identification of the cortical region under study.

After identification of the SIII locus as the most posterior or most postero-lateral of the three regions of activity, small amounts of [^{3}H] leucine were injected into the cortex at that site and the brains prepared for the autoradiographic tracing of connections.

Small injections of [^{3}H] leucine made in the SIII focus and containing 2 - 3 μCi of activity, labelled an area of cortex approximately 600 μ^2. Generally these injections lay 1 - 3 mm above the anterior suprasylvian sulcus usually at a level approximately opposite the lateral margin of the ansate sulcus. They occupied part of a cortical area in which layer V is dominated by large pyramidal cells having somatal diameters in the range of 30 - 40 μ. This area corresponds to area 5a (area praeparietalis gigantopyramidalis) of Hassler and Muhs-Clement (J. Hirnforsch., 6:377, 1964).

Injections that labelled all layers of the cortex led to axoplasmically transported label in a particular part of the ipsilateral thalamus. Single injections caused 3 or more small burst-like clusters of terminal labelling in a small-celled region lying in close apposition to the dorsal border of the ventrobasal complex (VB) but clearly separate from VB proper. It is suggested that this region may form a zone of further somatic sensory representation in the thalamus that projects preferentially to SIII. (Supported by grants from the Medical Research Council of Canada and the U.S. National Institutes of Health)

1310 EFFECTS OF DUAL STIMULATION OF THE SCIATIC NERVE OR THE SPINOTHALAMIC TRACTS ON THE THALAMIC PAIN CODE. R. Emmers. Department of Physiology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

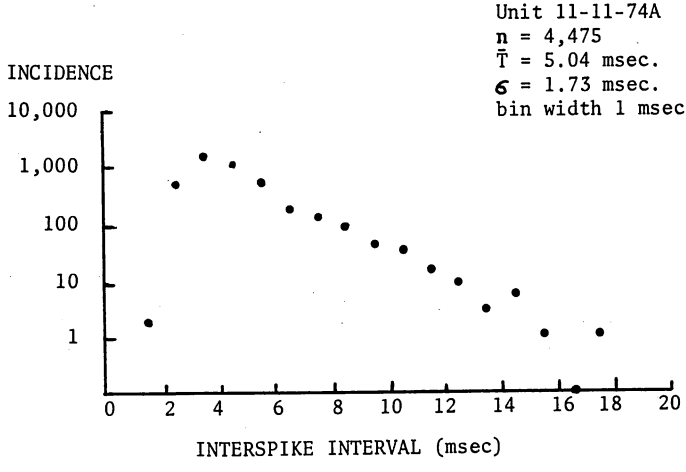
Recently (Brain Res. 103:425, 1976), it has been demonstrated that single thalamic neurons which relay noxious stimulation emit a specific temporal activity pattern not seen with neurons processing excitations of sensory modalities other than pain. Experiments of that study, however, utilized stimuli of very short duration, and, therefore, they could not illustrate the neurophysiological basis of continuous pain. To gain understanding of the latter, additional work was undertaken using repetitive stimulation. Rats anaesthetized with a mixture of chloralose and urethane were prepared for the recording of unit activity in the posterolateral portion of the thalamic SII and stimulation of the sciatic nerve contralaterally. The spinothalamic tracts (STT) were stimulated ipsilaterally to the recording site. The unit activity was analyzed by comparing spike density histograms compiled under various stimulus conditions on a 500 msec time base and 1000 repetitions of phase-locked stimulation. As described in Brain Res. 103:429, single stimulus pulses applied to the sciatic nerve once every two seconds reorganized the spontaneous, haphazard firing of an SII pain neuron in a specific temporal pattern characterized by several peaks on the density histograms. A short-latency peak coded the intensity of stimulation (I), whereas three clearly distinguishable late peaks (M_1, M_2, M_3) coded the modality of afferent activity. Dual stimulation of the sciatic nerve at various interstimulus intervals revealed certain principles about the organization of the activity peaks: 1) Each I peak was followed by its own M peaks; therefore, interlacing of the various peaks was obtained at some interstimulus intervals of dual stimulation but not at others. 2) The second stimulus of a pair was ineffective in firing the SII neuron at 40 to 60 msec interstimulus intervals. At greater than 60 msec intervals the I peak of the second stimulus progressively increased in height resulting in a build-up of the appropriate M peaks. 3) Because the SII neuron could not be fired at 40 to 60 msec interstimulus intervals, the peaks of the pattern could not be leveled by repetitive stimulation. 4) Although the M peaks were produced mainly by the activity of a positive feedback loop which connects the SII neurons with the CM-Pf complex, activity of the STT raised the firing probability of the SII neuron not only for the I peak but also for the M peaks. This was demonstrated by stimulation of the STT instead of the sciatic nerve. Consequently, repetitive stimulation of afferents produces a complex temporal firing pattern of single thalamic pain neurons which is a modified multiple of the basic pattern generated by individual stimuli applied at relatively long intervals. (Aided by grant NS-03266 from NINCDS).

1311 DISCHARGE PATTERNS OF HAIR AFFERENTS DRIVEN BY CONTINUOUS AIRJET STIMULATION. M.D. Goldfinger and V.E. Amassian. Dept. of Physiology, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203.

In cats under pentobarbital anesthesia, individual primary hair afferent fibers were recorded in the ipsilateral dorsal columns near C1 by glass-insulated tungsten microelectrodes. Such fibers have no resting activity, small (1cm^2) receptive fields, and followed 1:1 electrocutaneous stimulation with a 500 Hz train lasting 50 msec. The airjet is delivered from a fixed, 15 gauge hypodermic tube situated 2 inches or less from the receptive field on the forepaw. Maximum mean jet pressures of approximately 6mm Hg were recorded by a Statham (P23Db) gauge with the diaphragm exposed and at a distance of 40mm from the tube. With airjet stimulation, hairs are bent and exhibit a superimposed quivering movement.

Confirming unpublished observations of Giblin & Amassian, continuous airjet stimulation causes an irregular spike train in individual primary afferent hair fibers. Such afferents show marked differences in the mean interspike intervals during maximal steady-state discharge. In 33 units, 73% had mean intervals ranging from 4.5 to 14.7 msec (coefficient of variation = 0.2-0.77); mean intervals in the remaining units ranged from 20 to 90 (cv = 0.48-0.88). Regardless of the value of the mean interval with maximal stimulation, reducing the intensity of the airjet increased the mean interval (e.g. from 6.3 to 48 msec; from 35 to 51 msec). In brief mean interval units, after a short (1-3 msec) deadtime followed by a delay to peak, the declining phase of the interspike interval histogram approximates a single exponential (see Figure below). Long mean interval units show similar deadtimes during maximal stimulation, but have a slowed rising phase and more complexity in the declining phase of the interval histogram.

Independence of most consecutive intervals is suggested by the symmetry of most points around the 45° line on the joint interval scatter plot. No periodic discharges were revealed by the expectation density function (ED). However, the flat portion of the ED is attained in several ways: 1) A fast rise lasting 2-4 msec occurs, sometimes overshooting into a supranormal phase lasting 1-3 msec; 2) a slow, monotonic rise occurs lasting 10-40 msec. When maximally driven, brief mean interval units show ED pattern (1), but may show either (1) or (2) when weakly driven. However long mean interval units show pattern (2) for all intensities of stimulation. (Supported by USPHS, NIH Grants NS11219 and 10987).



1312 FINE STRUCTURE AND FUNCTION OF THE BILL TIP ORGAN IN GEESE.

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The bill tip organ in the beak of geese, representing a complex sensory structure in which different kinds of receptors are assembled in specialized tissue formations and tactile papillae was studied using light-, electron-, and fluorescence microscopy and electrophysiological methods. A single tactile papilla is innervated by 40 to 100 myelinated fibres of which 60 to 85% are thicker than 3 μ in diameter. Of the large myelinated fibres 40 to 55% innervate single Herbst and Grandry corpuscles located in the proximal and middle parts of the papilla, respectively. The other fibres mostly ascend towards the distal papilla, branch frequently and terminate on Merkel-type cells below the epidermis. Synaptoid junctions between these cells and the nerve terminals are abundant.

In the Grandry corpuscle the nerve fibre forms plate-like extensions which either lie as branched parallel terminals or as in series terminals between the Grandry cells. In spite of extensive searching and in contrast to previous reports no synaptoid junctions between nerve terminals and Grandry cells could be verified. There are, however, desmosomal attachments between each apposing nerve terminal and Grandry cell. The cytoplasm of the latter contains dense core granula but fluorescence microscopy so far has failed to identify catecholamines in them.

Ruffini endings, attached to strands of collagenous fibres, were seen below the horny plate and in the papillae. In the latter they are frequently accompanied by a single Merkel-type cell seemingly innvervated by the same nerve fibre as the Ruffini ending itself.

Most of the small myelinated fibres travel towards the tip of the papilla and terminate after branching with free nerve endings in the epidermis. They resemble other thermoreceptors and correspond to thermoreceptive units recorded electrophysiologically.

The vascular system was found to be remarkably specialized. A central artery ascends towards the tip of each papilla while a capillary and venous system below the epidermis surrounds the mechanoreceptors in the core of the papilla. The blood flow in this "fountain system" is controlled by two types of arterio- venous anastomoses located in the basal and lower portions of the papilla. Here a great number of adrenergic nerve endings could be demonstrated with electron- and fluorescence microscopy. A constant temperature of the tactile receptors inside the papilla at varying external temperatures appears to be maintained by changes of the blood flow in the vascular "fountain system".

Electrophysiologically two types of mechanoreceptive units with rapidly adapting discharge properties appear to derive from Grandry- and Herbst corpuscles, respectively. Slowly adapting units could not yet securely be differentiated into two types but it can be assumed that both Ruffini endings and the Merkel-type receptors give rise to them.

1313 CELLS OF ORIGIN OF EFFERENT PROJECTIONS FROM THE MONKEY FIRST SOMATIC SENSORY AREA. E. G. Jones, Dept. Anat. and Neurobiol., Sch. Med., Washington Univ., St. Louis, Mo. 63110

The areal and laminar distributions of cells giving rise to the major efferent connections of the first somatic sensory cortex (SI) were investigated in squirrel monkeys and Cynomologous monkeys. Single or multiple injections, each consisting of 0.05-0.1 µl of 50% horseradish peroxidase were made in the thalamus, striatum, dorsal column nuclei, spinal cord and in other areas of the cerebral cortex. Survival periods were 1-2 days and frozen sections of the brains were incubated in hydrogen peroxide and diaminobenzidine and counterstained with thionin.

Cortico-bulbar and cortico-spinal axons arise from larger pyramidal cells with somatal diameters in the range of 15-30 µ. Such cells are situated exclusively in layer V and are found in groups in subareas 3, 1, and 2 of SI. Approximately equal concentrations of cortico-spinal cells were found in these areas but the majority of cortico-bulbar cells lay in areas 1 and 2 or in the motor cortex (area 4).

Cortico-striate axons arise from smaller pyramidal cells lying primarily in layer V. These have somatal diameters in the range of 10-15 µ and are found in all subareas of SI.

Cortico-thalamic axons arise in large numbers from the modified pyramidal cells of layer VI in all subareas but a few larger pyramidal cells of layer V also appear to contribute to this projection.

Shorter ipsilateral cortico-cortical axons passing between areas 3, 1, and 2 of SI, between these areas and the second somatic sensory area (SII), and between the three areas and areas 4 or 5, arise from pyramidal cells of all sizes in layers II and III. After single small injections in other areas, labeled cortico-cortical cells form narrow, compact vertical groupings in areas 3, 1, and 2. A few longer cortico-cortical axons passing to areas 4 or 5 appear to arise from pyramidal cells of layer V.

Commissural axons arise from dysjunctive clumps of large pyramidal cells of layer IIIB in areas 3, 1, and 2 and pass to the homotopic area 3, 1, or 2 and to the contralateral SII.

The differential laminar distribution of efferent cells will be compared with the terminal distribution of the various afferent fiber systems terminating in SI, as determined by complementary autoradiographic studies. (Supported by NIH Grant number NS 10526.)

1314 A DOUBLE REPRESENTATION OF THE BODY IN "PRIMARY SOMATOSENSORY CORTEX" ("SI") OF PRIMATES, J. H. Kaas, M. M. Merzenich, C. S. Lin and M. Sur*
Departments of Psychology, Anatomy, and Electrical Engineering, Vanderbilt University, Nashville, TN. 37240, and Coleman Memorial Laboratory, University of California, San Francisco, California.

The idea of a single systematic representation of the body surface within the cortex of the postcentral gyrus of primates and the observation that this same region contains clear architectonic subdivisions are two longstanding but incongruent concepts that have been somewhat reconciled by the hypothesis of functional belts within a single body surface representation. Recently, however, a part of the "primary somatosensory field" ("SI") in the macaque monkey has been shown to include two separate, complete, and functionally distinct representations of the hand, with one representation within Brodmann's Area 3 (koniocortex), and the other, within Area 1 (Paul et al., Brain Res., '72). These partial findings suggested that the complete body surface may be represented twice in separate cytoarchitectonic fields within the classical "SI" of at least some primates.

To define the organization of the postcentral cortex more completely, we extensively mapped the "SI" region with microelectrodes in eight owl monkeys, (Aotus trivirgatus). The owl monkey was chosen for study principally because the central sulcus is shallow or absent in this species. In addition to microelectrode mapping studies, the tracers HRP and tritiated proline were injected at physiologically defined loci within the different cytoarchitectonic fields of "SI" in these monkeys. Among the results were the following: (1) There are two large highly-ordered representations of at least most of the body surface within "SI", each occupying a cytoarchitectonic zone (Area 3 or Area 1). (2) While the two body surface representations are similar, they are not exact replications of each other. For example, in the smaller posterior area the hairy skin of the fingers is represented over a restricted band of cortex within the glabrous skin field, while in the rostral area the representation of the hairy skin of the fingers is split to occupy cortex just medial and just lateral to the representation of glabrous skin. (3) The representation of the hand in Area 1 differs from that described in macaque monkeys. In the macaque, the tips of the fingers in both Areas 1 and 3 are represented along the rostral borders of the field, i.e., the two hands are represented serially. In the owl monkey, the finger tips are represented along the caudal border of Area 1, and along the rostral border of Area 3, i.e., the two hands are roughly mirror images of each other. (4) Neurons in both representations were activated by cutaneous stimulation. No specific joint afferent input was encountered within penetrations in either field. (5) Receptive fields defined for neurons in Area 1 were consistently larger than those for corresponding skin loci in Area 3. (6) Tracer studies suggest that the nucleus ventralis posterior lateralis (VPL) may be subdivisable since HRP injected into Area 3 labeled neurons in only the caudal portion of VPL.

These results, together with those of Paul et al. ('72) suggest that a dual representation of at least most of the body surface in Areas 3 and 1 of "SI" may be widespread in primates, emphasize that longstanding definitions of "primary somatosensory cortex" ("SI") must be redrawn for primates, provide evidence for a functional subdivision of VPL in primates, and raise questions as to the homologue of the single representation termed "SI" in other mammals.

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1315 DISCRIMINATION AND SCALING OF SENSATIONS EVOKED BY RADIANT STIMULI OF CONSTANT TEMPERATURE. Robert H. LaMotte. Dept. Physiology, The Johns Hopkins Univ., Medical School, 725 N. Wolfe St., Baltimore, Md. 21205.

These experiments demonstrate that the magnitude of warmth and thermal-pain sensations depends not only upon the surface skin temperature but also upon the intensity and time course of previous stimulations at the same locus on the skin.

Constant temperature pulses of radiant heat were delivered by a CO_2 infrared laser, with closed-loop control via a radiometer which remotely sensed skin temperature. Temperature steps of 2 to 12°C above a 38°C background were delivered to the thenar eminence of the hand of human subjects for 3 sec. Interstimulus intervals (ISI's) averaged 25 sec. Rise rates of the stimuli were faster than 30°C/sec. and the temperature over the 7.7 mm test spot could be maintained to better than $\pm$ 0.1°C. In one series of experiments, stimuli were delivered to one locus only. In another series, designed to achieve a long ISI, stimuli were delivered to 9 different loci rotated so that about 3.7 minutes elapsed between stimulations of the same spot. A two-alternative, forced-choice procedure measured detection thresholds for warmth. This choice, made by the subject on each trial, was followed by a choice of one of six labelled categories designated respectively as warmth, heat, or pain that was faint, moderate, strong or very strong. Frequency distributions of category choices were analyzed to locate category boundaries along a constructed scale of subjective thermal intensity. Differences between the means of the distributions provided a measure of discriminative capacity. Results showed that the perceived magnitude of each stimulus was less for short than for long ISI's and the percent correct in the detection task was lower for the shorter ISI. However, the capacity to discriminate between adjacent temperatures was the same for both short and long ISI's. There was a slight but consistent effect of a strong vs. a weak preceding stimulus temperature in lowering the perceived magnitude of subsequent stimuli delivered at the same spot. Thus, repeated delivery of stimuli to the same locus produced a temporal suppression of the perceived magnitude of all thermal stimuli by shifting the entire psychological scale but did not affect discriminative capacity. Analyses of the time course of temporal suppression indicated the greatest suppression at shorter intervals but less than maximal recovery even after 3 minutes. No consistent temporal interaction was observed for stimuli delivered successively to different loci on the skin. Raising the background temperature from 38°C to 43°C reversed the suppression and often produced primary hyperalgesia.

1316 SOMATOTOPIC AND LAMINAR ORGANIZATION OF SPINAL INTERNEURONS RECEIVING CUTANEOUS INPUTS FROM CAT HINDLIMB. Alan R. Light* and Russell G. Durkovic, Dept. Physiology, Upstate Med. Ctr., Syracuse, N.Y. 13210.

In anemically decapitate cats spinalized at T-8, three recording sites were located physiologically in the lumbar region of the spinal cord. The sites were: (1) the maximum cord dorsum N_1 wave of the cutaneous saphenous nerve (Saph.); (2) the maximum N_1 wave of the cutaneous superficial peroneal nerve (S.P.); (3) the region in which the two N_1 waves overlapped maximally (M.O.). Extracellular recordings were made from a total of 370 single interneurons in these three regions. Adequate stimuli, central delay to spike onset and receptive field area and location to touch, pressure and pinch were determined for each unit. Recording sites were marked by deposition of fast green. The data support a fairly discrete somatotopic representation of the hindlimb in the spinal cord with a columnar arrangement of cells with similar receptive fields. When the receptive fields to light touch were determined, no differences were observed in receptive field areas among cells in different spinal cord laminae at any of the recording sites. Instead, receptive fields with similar hindlimb locations and areas were distributed in nearly vertical columns in laminae 1-7. This columnar arrangement is depicted in Fig. 1B for units with the receptive field shown in Fig. 1A. However, laminar differences were observed when other parameters were investigated. In the Saph. zone, more intense stimuli were encoded by cells in laminae 1, 5, 6, and 7, while non-noxious stimuli were encoded by lamina 4 cells. The receptive field area to the most intense stimuli tended to be larger in more ventral cells than in more dorsal cells. Finally, the central delay was shortest in lamina 4, longer in 5, and longest in 1, 6 and 7. The M.O. zone did not exhibit such clear laminar organization. While lamina 1, 5, 6 and 7 encoded more intense stimuli than lamina 4, no laminar trends were observed in either receptive field area to intense stimulation, or in central delay. In the S.P. zone there was even less evidence of laminar organization. While lamina 1 cells responded to intense stimuli, no laminar distribution of adequate stimuli or receptive field area was observed. Unlike the Saph. zone, lamina 5 cells of the S.P. zone tended to have a shorter central delay than lamina 4 cells. Some cells in these regions were filled intracellularly with horseradish peroxidase and their dendritic fields and axon trajectories were observed. An example of a camera-lucida drawing made from one of these cells is shown in Fig. 1C. These data imply a complex organization of the spinal cord grey matter and help to explain the discrepancies between similar studies by Brown, Fuchs, and Tapper, J. Neurophysiol. 38, 19-25 (1975) and the observations of Wall, J. Physiol. 188, 403-423 (1967).

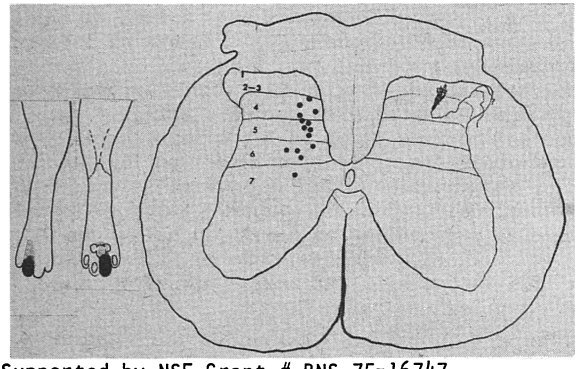

Fig. 1. A demonstrates the receptive field to light touch for the units plotted in B. The density of the stippling indicates the number of units with similar representation. In B, each dark circle represents one unit location. The data were collected from a total of 5 cats. C is a camera-lucida drawing of a cell filled with HRP. Its receptive field was dorsal ankle.

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1317 ORIGINS OF CORTICAL PROJECTIONS TO CERVICAL AND LUMBAR SPINAL CORD IN MONKEY. E.A. Murray* and J.D. Coulter. (SPON: R. Feinstein). Marine Biomedical Institute and Depts. of Physiol. & Biophy. and Psychiat., Univ. of Texas Medical Br., Galveston, Texas 77550.

In monkeys (M. mulatta), cortical neurons projecting to the spinal cord were identified using retrograde labeling with the enzyme horseradish peroxidase (HRP). In different animals, a single injection (<0.1-0.5 µl) of a 50% solution of HRP in saline was made through a micropipette (dia., 25-50 µm) in the spinal grey matter of the lumbar or cervical enlargement. The animals were allowed to survive for 3 to 4 days before perfusion and histochemical processing of the tissue. In the cortex, labeled neurons were confined to layer V and included both small pyramidal cells of this layer as well as large Betz cells in the motor cortex. There was a tendency for labeled neurons to occur in clusters of from 3 to 8 cells separated by adjacent areas where only occasional labeled neurons were seen. Correlation of the locations of labeled neurons with cortical cytoarchitecture indicated that areas 4 and 6 of the precentral gyrus, areas 3a, 3b, 1 and 2 of the postcentral gyrus, area 5 of the posterior parietal lobe, and the area in the lateral fissure corresponding to the second somatic sensory area all have direct projections to the spinal cord. Small injections of HRP into different regions of the spinal grey matter led to differences in the distribution of labeled neurons in the cytoarchitectonic fields of the cortex. When HRP injections were centered in the ventral horn, the largest numbers of labeled neurons were located in area 4 and the immediately adjacent area 3a in the central sulcus where electrophysiological studies show the distal hand and foot to be represented in the motor cortex. On the convexity of the hemisphere, fewer labeled cells were found in the rostral part of area 4 and none could be identified in the adjacent area 6. However, on the medial aspect of the hemisphere, extending into the cingulate sulcus, large numbers of labeled neurons were found in area 6 in the region identified by stimulation studies as being the supplementary motor area. With HRP injections in the ventral horn, there were always neurons labeled in each of the somatic sensory areas, but these were relatively few in number. On the other hand, when HRP injections were confined to the spinal dorsal horn, relatively greater numbers of labeled neurons were found in the somatic sensory areas 3a, 3b, 1 and 2, plus area 5. In contrast to the pattern of labeled neurons in the precentral gyrus, one or more distinct groups of labeled cells were seen in each of the cytoarchitectonic fields of the postcentral gyrus. The pattern of labeling showed multiple bands of neurons running medial to lateral, parallel to the central sulcus. Comparison of locations of labeled neurons with HRP injections in cervical versus lumbosacral spinal cord revealed a clear topographical organization to exist within the motor area 4, the somatic sensory areas, and area 5, with the lumbar cord represented medially and the cervical cord laterally in the hemisphere. In area 6, lumbar injections of HRP led to labeling of neurons adjacent to those found in area 4 on the medial aspect of the hemisphere, while cervical injections led to labeling of neurons more rostrally. Too few labeled neurons have been found so far in the region of the second somatic sensory cortex with injections in the lumbar cord to determine its exact topographic organization. These studies indicate that the corticospinal tract originates from cells of cortical layer V in the primary motor and somatic sensory areas, the second somatic sensory area, the supplementary motor area, and part of the parietal association cortex. In view of the distinctive response properties of neurons in these different cortical regions and their differential projections to parts of the dorsal and ventral spinal grey matter, the corticospinal system must have multiple functions in descending motor and sensory control. Supported by NIH grant NS 12481.

1318 DEVELOPMENT OF SENSORY NERVES AND NERVE ENDINGS IN THE MOUSE ORAL PALATAL MUCOSA. Pedro B. Nava, Jr., Dept. of Anatomy, The Milton S. Hershey Medical Center, Hershey, PA 17033

A preliminary light and electron microscopic study revealed the pattern of sensory innervation in the oral palatal mucosa of adult CRL/CD-1 mice (Nava, 1975). A unilateral, segmental innervation of the anterior palate by each major palatine nerve and the partial unilateral overlap in innervation by the minor palatine with the corresponding major palatine nerve was observed in the posterior portion of the palate. Transverse and longitudinal histological sections and confirmatory electron microscopic findings revealed the following sensory nerve endings and their distribution: taste buds on the medial aspect of the nasopalatine duct, along the Geschmachsstreiffen (taste strip), and soft palate; Meissner-like (dendritic bulboid) endings were concentrated in the apices of each palatal swelling along the transverse palatal rugae; simple encapsulated endings were noted in the anterior region of the palate; Merkel endings were observed in the basal layer of the oral epithelium adjacent to the Meissner-like endings in the connective tissue papillae; and free nerve endings were found throughout the palatal mucosa. The sequence and differentiation of sensory nerves and nerve endings was determined by using staged prenatal and postnatal animals. At 13 days prenatal, 1.5 to 2.0 days prior to palatal closure, nerve fibers were present in the posterior region of the palatine shelves. The nerves were accompanied by blood vessels and surrounded by mesenchyme. The nerves were not in contact with the epithelium of the palatine processes. At 15 days, just after palatal closure, the nerves made contact with the palatal epithelium. The transverse palatal rugae which contained corpuscular sensory endings in the adult, appeared to have developed at these points of contact. Cellular aggregations occurred in the palatal rugae in association with the neural outgrowths during the 16th day of development. These aggregations were a heterogeneous accumulation of cells composed of nerve fibers, Schwann cells, mesenchymal cells and blood vessels. Taste buds, the first recognizable receptors were seen in their adult locations on day 18. Except for the increase in branching of the nerve fibers, the presumptive corpuscular endings appeared little changed from the earlier stages. By the 19th day of development the overall adult pattern of palatal innervation was established. The process of sensory receptor differentiation was incomplete and continued postnatally.

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1319 EMOTIONALITY-INDUCED ANTINOCICEPTION. John A. Rosecrans and William T. Chance. Dept. Pharmacol., Med. Col. Va., Richmond, Va. 23298.

Although the gate control theory of pain is well known, much of the research stimulated by its predictions has focused on the inhibition of ascending nociceptive information at spinal cord levels by increased somatosensory activity. An integral portion of this theory is the hypothesized existence of descending CNS control modulating the perception of nociceptive stimulation in accord with past experience and current CNS activity. Support for the existence of this centrifugal control system has come from reports of the blockade of pain perception during the high states of arousal prevalent in battlefield situations or sport activities. Electrophysiological demonstrations of the inhibition of neural activity in ascending spinal tracts by electrical stimulation of the cerebral cortex, subcortical areas, and descending spinal pathways have also been reported. Furthermore, it has been shown that electrical stimulation of the septal, hypothalamic, and midbrain areas produces an analgesic response to noxious cutaneous stimuli. To the present time, however, no behavioral demonstrations of the natural activation of the central control mechanism has been reported in experimental animals.

Using the radiant heat tail-flick procedure for the assessment of antinociception, we have obtained behavioral evidence suggesting CNS activation of this control system. Lesions of the septal area in rats have been shown to produce dramatic increases in arousal and emotionality. To examine the relationship of the resulting hyper-emotionality to the degree of antinociception, we compared tail-flick latencies in septal-lesioned and sham-operated rats. Beginning 2 days after surgery each rat was rated for emotionality on a 5 point scale and then tested for antinociception. The septal-lesioned rats exhibited both increased emotionality and increased antinociception, as well as significant positive correlations between these measures, across the first 4 testing days. The degree of emotionality and antinociception showed parallel decreases with each day of handling, suggesting that the potentiation of antinociception was produced by the hyper-emotionality and not directly by the lesion.

Since the dificulty in handling the septal-lesioned rats may have produced responses incompatible with the tail-flick reflex, we next investigated the effects of CNS arousal by conditioned fear on antinociception in normal rats. Fear was conditioned to the stimuli associated with the tail-flick procedure by shocking (0.8 ma) one group (n=8) of rats on the tail-flick apparatus (15 sec/day) for 7 days (2 day on-1 day off). Additional groups (n=8) served as backward conditioning and no-shock controls. Comparison of the tail-flick latencies (day 8) revealed the experimental group to have significantly longer latencies than either of the control groups. The backward conditioning control group also showed significantly longer latencies than the no-shock group. Thus the levels of antinociception were distributed across the groups in an order predicted by the amount of fear conditioned to the tail-flick procedure.

We are interpreting these demonstrations as collaborative evidence of the centrifugal control system in the gate control theory of pain. The evolutionary significance of this type of arousal-activated descending control is evident. It is not to any animal's advantage to have to stop and nurse its pain-producing wounds when it is involved in a life or death struggle. Since the radiant heat tail-flick procedure is used extensively in the evaluation of the analgesic properties of drugs, these demonstrations are also important to the area of pharmacology. If the tested compound or other experimental manipulations also elicit hyper-emotional states, the analgesic effects of the drug could be exaggerated. It is suggested that the degree of emotionality always be considered when any evaluations of antinociception are made using the tail-flick procedure.
(Supported in part by NIH grants PSH-DE00116; DA-00296-02)

1320 RESPONSE PROPERTIES OF VIBRISSA-RELATED NEURONS IN SmI NEOCORTEX OF UNANESTHETIZED RATS. D. J. Simons* and T. T. Sandel* (SPON: V. Hamburger). Department of Psychology, Washington University, St. Louis, Missouri (63130).

Using glass microelectrodes we have studied the responses of single neurons in SmI neocortex of unanesthetized paralyzed rats to deflections of their contralateral vibrissae. Specifically, we examined the size of receptive fields, the presence or absence of directional sensitivity, and the relations between unit discharges and specific aspects of vibrissal deflection. Although many cells respond, as expected, to deflection of an individual vibrissa only, a substantial proportion of neurons are activated by movement of two or more vibrissae. On the basis of our sample to date, such neurons appear to exist at all levels of the cortex. Many neurons are directionally sensitive; both excitatory and inhibitory response patterns have been observed. By using standardized stimuli, three major functional groups of neurons can be distinguished with respect to their responses to specific aspects of vibrissal deflection—units that respond primarily to a) transient stimulation (vibrissal movements), b) steady-state stimulation (fixed vibrissal displacement), or c) both transient and steady-state stimulation. For some cells the relation between neural response and peripheral stimulation is described by either linear or semi-logarithmic functions. Quantitative analyses also indicate that in the case of neurons that respond to deflection of more than one vibrissa, the vigor of response to a particular stimulus may differ depending on which vibrissa is stimulated. For these neurons the response patterns to deflection of different, individual vibrissae are qualitatively similar, but the strongest response is typically elicited when the "primary" vibrissa driving the cell is deflected in the cell's preferred direction. These data suggest that a) vibrissa-related neurons in the cortex encode information concerning stimulus direction, duration, velocity and amplitude and b) information from different contralateral vibrissae may be integrated in SmI neocortex of rat. (Supported by NIGMS Grant GM1900).

1321 ELECTRICALLY-INDUCED HYPALGESIA OF THE HUMAN DENTAL PULP. Richard B. Tacke*, R. Wayne Fields, Peter C. Sakellaris*, and Bhim Sen Savara* (SPON: John M. Brookhart). University of Oregon Health Sciences Center, School of Dentistry, Portland, Oregon 97201.

The present experiments test whether the electrical stimulation of oral mucosa (EA stimulation) produces attenuation of experimentally induced pain in the pulps of nearby teeth. Human subjects were used to provide a direct measure of perceptual effects (verbal report).

A series of experiments were conducted in which no EA was applied. The data from 53 sessions (84 trials/session) in 23 subjects indicates that the frequency of occurrence of a reported level of perceived intensity less than or more than that expected relative to multiple stimulus categories occurred in 8% and less than 1% of the trials, respectively.

The first EA experimental series involved an extension of work reported previously (Tacke, Fields, Sakellaris, and Savara, Neurosci. Abst. 1: 119, 1975). Our 'standard' EA waveform was employed, composed of a continuous train of bidirectional rectangular pulses at 100 pps and 50% duty cycle, at an intensity 10-20% below perceptual threshold (typically 0.5-1.0 ma). The data involved 32 subjects with 84 trials per experimental session and an average of 8 repeated sessions per subject. Pre-established levels of perceived sensation relative to stimulus intensity were reported to be reduced by all subjects during EA. Perceived intensity was below that expected in 93% of EA trials and only 17% of control (pre-EA) trials, a difference which was highly significant ($p < .001$). Of 5 categories ranging to moderate pain, EA-induced decreases in perceived intensity averaged 1-2 categories. Induction to full effectiveness required 5-20 minutes and recovery to baseline conditions following EA termination ranged from 2 to 3 hours.

The second EA experimental series involved amplitude modulation of the standard EA waveform. Four modulation waveforms were studied: 1), no modulation; 2), unidirectional square waves, 3), a unidirectional sawtooth waveform; and 4), a sine waveform. Modulation frequencies ranged from 0.1 Hz-10 KHz in order-of-magnitude steps. In 26 preliminary sessions, it was determined that there was no significant difference between waveforms and that the 1 Hz frequency was most effective. Subsequent experiments involved the use of a 1 Hz sine wave, 35 sessions using the modulated EA waveform (14 subjects) and 20 sessions using the unmodulated waveform (the same 14 subjects) to optimize data comparability. All sessions involved 84 EA stimulus trials. Perceived intensity was reduced below that expected in 93% of trials involving the unmodulated waveform and in 100% of trials involving the modulated waveform. The modulated and unmodulated waveforms differed significantly in two respects. First, maximal rates of occurrence of decreases in perceived intensity were achieved within an average of 5 minutes as opposed to 20 minutes using the unmodulated and modulated waveforms, respectively ($p < .001$). Secondly, after maximal rates of occurrence of decreases in perceived intensity had been attained, the average magnitude of downward shift in sensory categories was 3-4 as opposed to 1-2 for the modulated and unmodulated waveforms, respectively ($p < .001$).

Preliminary data is now available from two new experimental protocols. First, extraoral EA stimulation is being compared with intraoral application and results indicate that extraoral application of EA currents is superior. Secondly, tests on patients experiencing pathological orofacial pain are in progress and results establish that complete relief from pain can be attained after a short induction time.

1322 CAUSALGIA: INDIRECT MEASUREMENTS OF SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN PATIENTS AND MATCHED CONTROLS. Albert J. Tahmoush , Stephen Camp, * J. Richard Jennings.* Dept. of Exp. Psychophysiology, WRAIR, Washington, D.C. 20012

Causalgia is a clinical pain syndrome which occurs after trauma and is characterized by severe, steady, burning pain and hyperalgesia of the affected area. Signs of local sympathetic nervous system abnormalities are present, and sympathectomy frequently results in pain relief. These observations have led to the suggestion that causalgia is a reflex sympathetic dystrophy. This suggestion implies that causalgia results from increased SNS activity in the involved extremity.

The relationship between SNS activity and causalgia was studied by performing measurements of skin temperature, skin conductance, blood volume pulse and blood volume on the affected and homologous non-affected extremity of nine patients and matched controls. The values for each limb and the absolute value of an asymmetry index (affected limb minus non-affected limb) were analyzed.

For the initial analysis, differences in clinical treatment antedating the measurement period were not taken into account since all patients continued to have severe pain. Only two of the dependent measures showed significant difference between patient and control groups. The mean skin conductance values were lower and the skin temperature asymmetries were greater in the patients as compared to controls. Inspection of the asymmetries present in the patients revealed a relation to prior treatment. In three patients with no sympathetic intervention, the affected limbs showed a relatively consistent pattern of sympathetic hyperactivity. In the remaining patients who were treated with sympathetic blocks or sympathectomy without pain relief, the affected limbs generally showed a pattern of decreased sympathetic activity. Therefore, although SNS hyperactivity may occur in patients with causalgia, the chronic pain does not appear to be dependent on the presence of increased SNS activity in the affected extremity.

1323 USE OF WEIGHTING FUNCTIONS TO CHARACTERIZE INPUT-OUTPUT RELATIONS OF CUNEATE NEURONS IN CAT. J.P. Walsh*, M.B. Bromberg, and D. Whitehorn. Dept. Physiol and Biophysics, Univ. of Vermont, Burlington, VT 05401

Functional connections between neural populations can be quantitatively described using weighting functions. Here we examine the use of weighting functions (WF) to describe the response of cuneate neurons to graded levels of activity in their afferent input population. The time course of probability of cell discharge (described by the post-stimulus time histogram:PSTH) is a weighted sum of activity on input fiber groups,

$$P(t_i) = \sum_j w_j \cdot x_j(t_i),$$

where, $x_j(t_i)$ is the activity of the jth input fiber group at time t_i and w_j is the relative weight given to that activity. This expression represents a set of simultaneous equations which can be solved for the weighting function $\{w_j\}$ with matrix inversion techniques, if the PSTH and the time course of the input activity are known.

Ideally, the WF represents the strength and nature of connections between groups within an input population and a post-synaptic cell. Therefore it should predict cell response to a variety of input patterns. We have measured cell response (PSTH) as a function of input level, derived WFs based on maximal input conditions, and tested their predictive ability.

Measurements were made on 114 individual cuneate cells in 16 cats by stimulating the superficial radial nerve (SRN) at ten intensities while measuring the latency of all evoked spikes and the area under the compound action potential (CAP) on the SRN. Input CAP areas were sorted by size relative to the maximum, and the PSTH was determined for each input level. Input groups were defined on the basis of conduction time to the cuneate from the SRN. The distribution of conduction times was determined in a separate set of experiments using recordings from 205 individual SRN fibers. $x_j(t)$ was calculated from these data on the assumption that each input group influenced the PSTH according to the expression

$$x_j(t) = N_j \cdot [(t - T_j)/\tau]^3 \cdot \exp[1-(t - T_j)/\tau],$$

where N_j and T_j are the number of fibers and the conduction time for the jth input group and τ is a time constant. For each cell, a WF was calculated from this input function and the experimentally obtained PSTH at the maximum input level.

To produce simulated PSTHs comparable with their experimental counterparts, input activity vs. CAP area was estimated with a CAP simulation. The the WFs were used to produce simulated PSTHs by calculating membrane depolarization as a weighted sum of this input activity. This depolarization was superimposed on a noisy resting potential, and a threshold was introduced.

Experimentally we observed heterogeneity among cells. Differences in the PSTH at maximum input were evident as well as a variety of patterns in the matrix describing the PSTH as a function of input level. Differences in input level required for activation, and steepness of response change with input level change also occurred.

The weighting function included both positive and negative elements. The negative elements compensate for the absence of threshold in the WF derivation. They minimize input contributions at times when the probability of cell discharge is zero. Nevertheless, WFs derived in this manner successfully predicted PSTHs for maximal input in a simulation that included threshold.

The simulated PSTH vs. input CAP area matrices fit the experimental data well around maximum input. The simulated results showed threshold phenomena, and increased latency and variability around response threshold, but did not agree in detail with the experimental data at every input level. This lack of agreement is probably due to the use of only maximum input data in deriving the WFs. (Supported by NS09472, NINCDS; and PHS 5 T01 GM00439-15)

1324 TOPOGRAPHY OF TYPE I MECHANORECEPTOR RESPONSES TO MOVING STIMULI. W.J. Williams and F.J. Looft* Bioelectrical Sciences Laboratory, University of Michigan, Ann Arbor, MI 48109.

Adult cats, anesthetized with sodium pentobarbital, were prepared for single unit recording from peripheral nerves subserving the hind limbs. The nerve was split and fibers were elevated on silver electrodes in a mineral oil pool.

A computer controlled punctate stimulator was used to apply constant force linear stimuli to the hairy skin of the leg. The linear stimulus was moved back and forth over the skin at a constant velocity.

We were able to map the response of the receptors as a function of stimulus position by dividing the stimulus duration in each direction (the +x and -x directions) into a preset number of consecutive bins, and by recording the number of impulses produced by the receptor for each bin. Area maps were produced by averaging several sweeps of the stimulator in one y position followed by an index of y position. The scan-index routine was repeated until a 1-cm square area was mapped. The responses of the receptors in the forward (+x) and reverse (-x) directions were kept separate for comparison.

The response of numerous cutaneous units to manual stimulation was observed. The response types were usually in harmony with the classifications established by Burgess et al. (J. Neurophysiol. 31:833, 1968). Thirty one units were studied thoroughly using the x-y stimulator. Eight units conformed to the description of type I mechanoreceptors. Other receptor types such as field and type II mechanoreceptors were observed as well. Hair receptors were observed frequently, but their responses were not amenable to the present stimulator configuration. Since the most clear-cut and interesting responses were obtained from type I mechanoreceptors we will confine our observations to this type.

The approach provides a clear view of the topography of type I mechanoreceptor responses. Typical responses are shown in Fig. 1. Multiple response foci are common and each focus exhibits a similar response topography.

The topography of a given focus can be commonly described as being a hillock with ellipsoid cross sections. The major axis of the ellipsoid is somewhat exaggerated compared to the minor axis. The direction of orientation of the long axis varies and may be, in fact, orthogonal to the direction of orientation of the long axis of another focus belonging to the same receptor. The effects of a number of different stimulus parameters such as velocity, force and direction were examined. Repeatability and adaptation tests were made. The response of the receptors was increased by increased force and increased velocity of stimulation, but the topography of the response was not altered markedly under these different conditions.

It seems that information concerning stimulus orientation is available at the receptor level. This does not preclude additional higher level processing as well, however. Supported by USPHS Grant NS 08470-7.

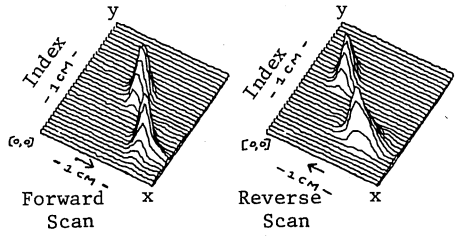


Fig. 1 Topography of a Type I Mechanoreceptor (32x32 bins) Each x line is 10 averaged responses to a 2 cm/s linear stimulus. A force of 1 gram (wt) was applied via a 0.5 mm rolling ball

1325 FLUTTER AND VIBRATION CHANNELS: ANALYSIS OF MASKING USING THE THEORY OF SIGNAL DETECTION. A. Zambelli*, T. Bando and W.A. Spencer* (SPON: E. Gardner), Div. Neurobiol., Dept. Physiol., Coll. of Phys. & Surg., Columbia U., New York, N.Y. 10032

Specific submodality representations within the primary somatosensory pathways have been identified by others through the use of stimuli specific for particular receptor types. However, the extent of central interaction within a particular submodality or between different submodalities has not been worked out in detail. The phenomenon of cutaneous masking is a useful tool in the investigation of such interactions. It is essential in the study of masking, however, that one differentiate between changes which result from alterations in the discriminative capacities of the sensory system (d') and those which result from shifts in the subjects' response criterion. The application of the Theory of Signal Detection allows for such an analysis.

In the present study the Theory of Signal Detection was applied to the examination of masking of cutaneous sensations evoked by sinusoidal mechanical skin indentation of low frequency (i.e. flutter) or high frequency (i.e. vibration) in human subjects. Simultaneous presentation of stimulus trains or single cycles in a two stimulus paradigm was used. Three experimental conditions (flutter vs. flutter, vibration vs. vibration, and vibration vs. flutter) were examined at various sites on the forearm, wrist and hand. The interstimulus distances employed were the minimum separations at which subjects could easily discriminate conditioning and test stimulus loci; and low amplitude indentation ranges were chosen to minimize chances of coactivation of the two systems by either one of the stimuli. Subjects were required to rate their subjective estimate of the probability of occurrence of a particular test stimulus utilizing a maximum of five rating categories. The rating procedure is extremely efficient, allowing the calculation of the probabilities which correspond to varying criteria, and thus generating a number of data points on the empirical ROC curve.

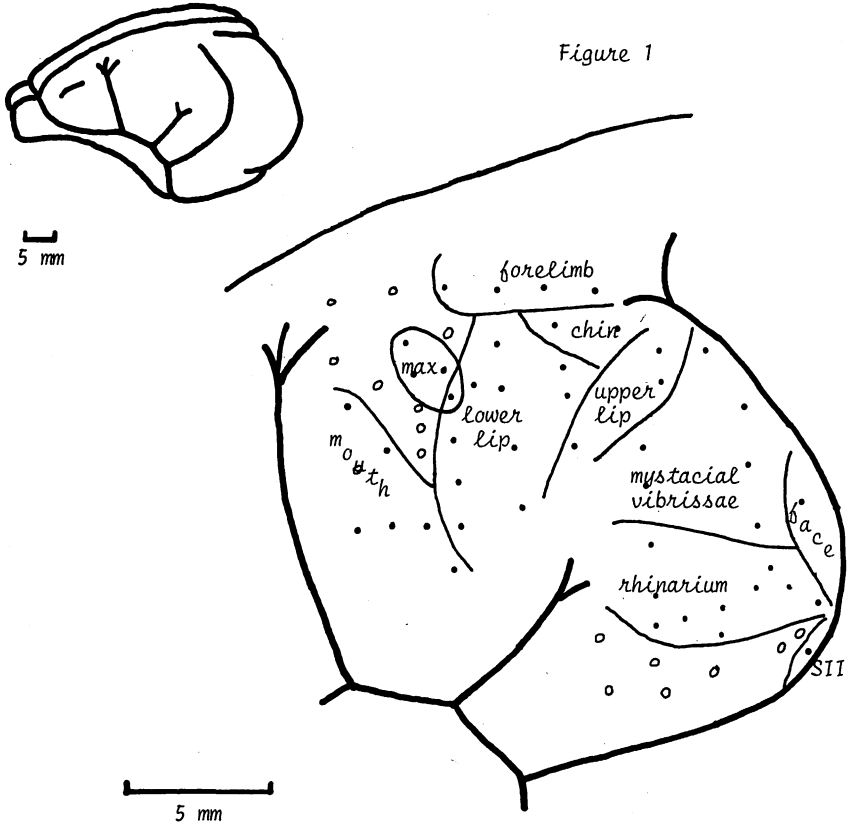
Maximal masking was obtained within the flutter and vibration submodalities themselves. This masking represented alterations in the discriminative capacities of the sensory system as supported by significant differences in d' obtained in each experimental and control condition. The masking by the vibration submodality of the flutter submodality was less pronounced than the masking within these systems. In other words, the results indicate considerable masking interaction within the flutter and vibration submodality channels themselves, but a much weaker interaction between different specific submodality channels. This application of the Theory of Signal Detection supports, in all cases that we studied, the notion that masking, as defined in the present experiment, results from some alteration in the discriminative capacities of the somatosensory system and not from criterion shifts on the part of the subject during presentation of the masking paradigm.

(Supported by 2 RO1 NS 12744 NEUA)

1326 SINGLE REPRESENTATION OF MYSTACIAL VIBRISSAE IN SI NEOCORTEX OF RUFOUS WALLABY THYLOGALE BILLARDIERII. W. Lee Weller, John R. Haight*, Lee Neylon* and John Irwin Johnson. Dpt. Anat., Univ. Tasmania, Hobart, TAS., AUSTRALIA

In the primary somatic sensory (SI) neocortex of American opossums Didelphis virginiana, Pubols et al. (1976, J. Comp. Neurol. 165:229) reported two separate, mirror image, representations of the mystacial vibrissae, one on either side of the rhinarial projections. Using tungsten microelectrodes, we mapped electrophysiologically the corresponding projections in a larger, Australian, marsupial, the rufous wallaby or Tasmanian pademelon T. Billardierii, and found that the double vibrissa representation does not occur here and thus is not a general feature of marsupial brains: in Thylogale there is but a single representation of vibrissae in SI cortex, entirely medial to the projections from the rhinarium (Fig. 1). We also located the position of the second sensory (SII) cortex, not seen hitherto in wallabies (Lende, 1963, Science 141:730) and confirmed Lende's finding of a "misplaced" projection from hairy maxillary skin (max in Fig. 1) between the lower lip and forelimb projections, along with the pattern of other projections Lende found in the dama wallaby Macropus (Thylogale) eugenii. (Supported by research grants from ARGC and NSF GB 43236).

Figure 1



1327 RESPONSE PROPERTIES OF DENTAL PULP NEURONS IN TRIGEMINAL NUCLEUS CAUDALIS UNDER CHLORALOSE ANESTHESIA. Ronald F. Young. Dept. Neurological Surgery, Upstate Medical Center, Syracuse, New York 13210.

Extracellular unit recording was carried out in trigeminal nucleus caudalis of cats under chloralose anesthesia. Units were identified by their responsiveness to electrical stimulation of the ipsilateral upper or lower canine tooth pulp. The majority of units also responded to non-noxious graded mechanical stimuli applied to receptive fields on the face. A small fraction of the units responded only to tooth pulp stimulation. Units were recorded from .2 mm. rostral to the obex to 6.5 mm. caudal to the obex and were located primarily along the ventral medial border of nucleus caudalis in or adjacent to the lateral reticular formation. A few units were located in the magnocellular portion of nucleus caudalis. Response latencies and firing patterns were essentially identical to a group of units previously identified in a similar location in response to similar stimuli recorded under barbiturate anesthesia. Conditioning experiments using the dental pulp stimulus or the mechanical stimulus in various combinations as conditioning stimulus or test stimulus also gave results essentially identical to similar experiments carried out under barbiturate anesthesia. It has been previously noted that response patterns of trigeminal neurons elicited by dental pulp stimulation differed in the rostral portion of the spinal trigeminal nucleus of the cat, main sensory nucleus and nucleus oralis, as opposed to the firing patterns recorded in the caudal part of the nucleus, nucleus caudalis. These differences had been proposed by some as having functional significance for the coding of noxious and non-noxious stimuli. Because the former units had all been recorded under chloralose anesthesia and because the latter units had been recorded under barbiturate anesthesia, it was not possible to determine whether the observed differences in firing characteristics were a result of different anesthetic techniques or whether they reflected true differences in the neuronal populations. It appears from the present experiments that the latter is the case and that further investigations as to the significance of these differences is indicated.

1328 RESPONSE OF SINGLE UNITS IN THE PERIAQUEDUCTAL GRAY OF THE RAT BRAIN TO NOXIOUS STIMULI AND THE EFFECT OF ANESTHETICS ON THEIR FIRING CHARACTERISTICS. Michael M. Behbehani, Dept. Physiology, Sch. Med., Cincinnati, OH, 45267

Responses of single units in the periaqueductal gray area of the rat brain to noxious stimuli applied to the tail or any of the four limbs were measured. The cells in this region can be classified in three groups according to their firing characteristics. One group of cells responded by a short burst of firing with a latency of 10 to 30 m.sec. - that lasted for a period of 30 to 40 m.sec. followed by an inhibitory period of 200 to 250 m.sec. and a second burst of activity that lasted 40 to 60 m.sec.

The second group of cells responded to stimulation by excitation that lasted 200 to 300 m.sec. followed by inhibition that lasted 50 to 100 m. sec. The third group did not respond to stimulation.

The receptive field of the cells in the first and the second group was large and included the tail and the four limbs. Effect of anesthesia and morphine on the response of the cells in this region was measured. Morphine microinjected in this area substantially decreased or totally abolished the inhibitor phase. Similar results were obtained by addition of halothane to the anesthetic gas mixture.

1329 EFFECTS OF MICROINJECTED NARCOTIC ANALGESICS INTO THE PERIAQUEDUCTAL GRAY (PAG) ON THE RESPONSE OF RAT SPINAL CORD DORSAL HORN INTERNEURONS. G.J. Bennett* and D.J. Mayer. Depts. of Psych. and Physiol., Virginia Commonwealth U., Med. Col. of Va., Richmond, VA. 23298. (SPON: J. Hu).

Microinjection of narcotic analgesics (NA) into and focal electrical stimulation of the PAG have been shown to produce analgesia in several behavioral assays. It has been suggested (e.g., Mayer and Liebeskind, Brain Res. 68:73-93, 1974) that both of these manipulations activate a common neural substrate which actively inhibits the afferent pathway for pain. It has been shown that stimulation of PAG inhibits the response of spinal cord neurons to noxious stimuli. The present study sought to determine whether the mechanism of action of microinjected NA was similar. Adult rats with cannulae chronically implanted in PAG were microinjected with etorphine (0.25-0.50 µg, 1.0 µg/µl) and assayed for analgesia by the tail-flick method. In subsequent acute single unit recordings under light chloralose anesthesia in the same rats, nociceptive neurons were inhibited by microinjected morphine or etorphine in a large majority of cases. As little as 0.25 µg of etorphine was often sufficient to completely inhibit a unit's response to tissue damaging pinch or to radiant heat of 50°C. Non-nociceptive neurons were generally unaffected by microinjected NA. The onset and duration of etorphine inhibition paralleled that seen in the behavioral assay. The narcotic antagonist, naloxone (1 mg/kg i.v.), reversed the etorphine induced inhibition. These results suggest: 1) that the analgesia produced by narcotics is mediated, at least in part, by activity in a descending inhibitory system initiated in or near the PAG, and 2) that the analgesia produced by electrical stimulation of and microinjected NA into the PAG may result from similar effects on spinal interneurons. (Supported by PHS Grant DA 00576.)

1330 ROLE OF "WARM" FIBER DISCHARGE IN WARM DETECTION MEASURED BY FASTEST REACTION TIME IN RHESUS MONKEY. S. Berg*, R. Harris, R. Beitel, & R. Dubner, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD. 20014.

Contact thermal stimuli were presented to the upper hairy lip of two rhesus monkeys trained to perform a standard reaction time (RT) task. Warming stimuli starting from an adapting temperature of 35°C were varied for rate (1°C to 9°C/sec) and intensity (0.4 to 5.0°C) and randomly occurred 2-8 sec after the depression of a panel button. In initial experiments, release of the panel button within 3.0 sec from the onset of warming was rewarded with water (0.3 cc). Subsequently, the same animals were trained for the most rapid detection of the onset of the warming stimulus by narrowing the reward window until further restriction produced a more variable RT distribution and an increase in incorrect responses. Final reward windows ranged from 285 to 650 msec depending on the rate of temperature change. Restriction of the reward window produced decreases in average RTs at all rates. Shorter average RTs occurred in response to faster rates of change, but average RTs were unaffected by increase in intensity. Average RTs in the restricted window condition at 5°C/sec and 1°C/sec were compared with discharges of warm fibers innervating the lip region in response to identical stimuli. At both rates of temperature change, a significant increase in mean frequency discharge above resting level occurred about 200 msec earlier than the average RTs. The results confirm our previous conclusions (Neuroscience Abstracts 1:147,1975) that, 1) RTs to warming are determined mainly by rate of stimulus change and not stimulus intensity, and 2) warm fibers alone provide the critical neural input essential for warm detection from 35°C adapting temperatures in rhesus monkey.

1331 CEREBRAL MODULATION OF THE EXCITABILITY OF NEURONS IN THE CUNEATE NUCLEUS OF THE DOMESTIC CAT. Mark B. Bromberg and Arnold L. Towe Dept. Physiol. & Biophys., Univ. Wash. Sch. Med., Seattle, WA 98195

Extracellular microelectrode recordings were made throughout the extent of the cuneate nucleus in cats anesthetized with chloralose and paralyzed with decamethonium bromide. The hunting stimulus was shock to the dorsal funiculus at C3; testing stimuli were applied to the limbs, to the medial lemniscus and to the cerebral cortex. In a sample of 808 single neurons, 479 were adequately classified as relay (103) or non-relay (376), and 412 of these were also tested for modulation by pericruciate cerebral cortex. Three significant findings emerged: 1) many relay neurons were excited by cerebral stimulation, 2) some neurons were excited from one and inhibited from the other cerebral hemisphere and 3) many neurons were unaffected by cerebral stimulation. Among the relay neurons, 26% were directly excited by contralateral and 11% by ipsilateral cerebral stimulation, whereas the corresponding values for the non-relay neurons were 55% and 36%. Similar values for initial inhibition were 43% and 42% for the relay neurons, and 32% and 42% for the non-relay neurons. A period of decreased responsiveness often followed the cerebral excitatory effect; including this effect with the initial inhibitory effect increased the values for inhibition to 59% (contra) and 49% (ipsi) for the relay neurons, and to 75% and 69% for the non-relay neurons. Correlations in response latency to contralateral and ipsilateral cerebral stimulation were highly significant for both the relay and non-relay neurons, but no relationship was apparent between the response latencies to cerebral and C3 dorsal funicular stimulation. Mean response latencies to cerebral stimulation were clearly shorter among the relay than among the non-relay neurons. Cerebral modulation was somewhat more prevalent among MTJ-sensitive neurons than among touch-sensitive and hair-sensitive neurons. (Supported by NIH grants NS-05136 and NS-00396.)

1332 EVIDENCE FOR COMPLEX SENSORY NERVE ENDINGS IN AND AROUND RAT MOLARS. Margaret R. Byers. Anesthesiology and Center Research Oral Biology, Univ. of Washington, Seattle, 98195.

[^{3}H]-L-proline was injected at multiple sites into the right trigeminal ganglion of mature rats (n = 9) to allow rapid axonal transport of labeled protein into ipsilateral trigeminal nerve endings. Serial sections were made completely through the molar regions of right and left upper and lower jaws and prepared for autoradiography. Right molars (but not left) showed silver grains concentrated over three zones: the subodontoblast plexus (containing densely aggregated free nerve endings), the gingival epithelial cuff, and the coronal circumpulpal dentin. In the gingival cuff, free endings surround the molars adjacent to the enamel-cementum junction. Nerve endings in dentin spiral around odontoblast processes in the dentinal tubules, occurring in a graded frequency ranging from occasional tubules in intercusp regions to innervation of all tubules surrounding the occlusal surfaces of the molar cusps or ridges. Club-shaped endings were occasionally seen in the free gingiva. The few silver grains over periodontal ligament were associated with blood vessels or denoted nerve bundles traversing the region; rare silver grains indicated a few free nerve endings.

These results suggest that dental sensation in rat molars depends primarily on numerous nerve endings underlying the enamel (in the gingiva, coronal dentin, and coronal pulp). The diverse shape and distribution of these endings suggests sensitivity to other stimuli -- such as pressure -- as well as to pain. (Supported by NIH grants DE 02600 and GM 15991)

1333 EXCITABILITY OF THE INTRASPINAL PORTION OF PRIMARY AFFERENT C FIBRES IN THE SPINAL CORD OF THE CAT. O. Calvillo* (SPON: F. Roberge). Anaesthesia Research Department, McGill Univ., Montreal, Canada.

The excitability of the terminal region of cutaneous afferent nerves was tested in decerebrate cats by electrical stimulation with bipolar concentric electrodes inserted in the dorsal horn. The antidromic C fibre potentials, recorded from the sural nerve, had thresholds 20-40 times those of the most excitable fibres. C fibre conduction velocities varied between 0.8 and 1.5 m/s. Orthodromic C fibre activity induced by noxious heat applied to the foot reduced the amplitude of the antidromic potential, presumably as a result of collision, confirming that the antidromic potential travelled along sensory C fibres. Non-noxious stimulation of the foot, as well as electrical stimulation of the posterior tibial nerve activating A fibres only, sometimes increased the excitability of the intraspinal C fibres. Cooling of the spinal cord localized to the 8th and 9th thoracic segments with a Peltier device, permitted reversible spinalization of the animal, thereby abolishing the influence of descending pathways on the lumbosacral spinal cord. The excitability of the C fibres was reproducibly diminished during the spinalized state, returning to control levels upon rewarming the cooled segments. These observations suggest that in their intraspinal course the sensory C fibres come under the influence of supraspinal structures, and that the terminals may thus be maintained in a state of tonic depolarization, possibly controlling nociceptive input to the central nervous system.

1334 EFFECT OF SUMMATION IN THE SUBJECTIVE MAGNITUDE OF VIBROTACTION. Anthony J. Capraro* and Ronald T. Verrillo. Inst. for Sensory Res., Syracuse Univ., Syracuse, NY 13210.

The duplex mechanoreceptor theory is a successful model for explaining threshold phenomena in vibrotaction. The model states that vibrotactile information is carried by two discrete channels, one subserving high frequencies (Pacinian) and one low frequencies (non-Pacinian). The purpose of these experiments is to determine how the channels interact at suprathreshold levels of stimulation which must involve both channels. Hypothesis: The subjective magnitude of a suprathreshold stimulus which activates both channels results from an additive interaction between the two channels. We have attempted to measure the interaction using a stimulus which provides a determinable amount of activation to each channel. Subjects were asked to judge the total loudness of a stimulus pair which consists of a low-frequency vibration followed by a high-frequency vibration. Results indicate that the interaction between the channels is equivalent to an addition of the subjective magnitude in each channel, an effect we have termed summation. When the two stimuli of the pair were presented simultaneously, the results agree with experiments in which the two stimuli were sequential. Single frequency stimuli do not always activate both channels equally. Depending on the frequency, energy will be presented in varying proportion to the two channels and the data indicate that the addition of each channel's subjective magnitude becomes nonlinear when the two channels are not equally activated. Finally, the measured summation effects will be used in conjunction with data calculated from the duplex mechanoreceptor theory to predict some suprathreshold phenomena. Data will be presented which tests the accuracy of these predictions.

1335 SOURCES OF AFFERENTS TO THE LATERAL CERVICAL NUCLEUS IN CAT AND DOG. Arthur D. Craig, Jr.*(SPON: R.D. O'Brien). N.Y. State Coll. Vet. Med. Cornell University, Ithaca, NY 14850

Electrophysiological investigations of the lateral cervical nucleus (LCN) in cat and dog have produced results apparently at variance with the anatomical data presently available(Ha & Liu 1967)on the location of cells projecting to the LCN, specifically with regard to forelimb and proprioceptive inputs. In this study neurons labeled with horesradish peroxidase (HRP)were observed in spinal cord and medulla of young dogs & cats, following injections of 0.1-0.4μl of 20% HRP into the LCN through fine glass pipettes. Approach was made to the LCN either dorsally through the dorsolateral funiculus or medially through the dorsal column & dorsal horn, at mid-C_1 or mid-C_2 or both. In both dog & cat spinal cord, HRP-labeled cells were found predominantly in ipsilateral lamina IV(n. proprius)in cervical, thoracic, & lumbosacral cord; the highest desnity of labeled cells was found at C_{7-8}(possibly due to propriospinal contributions). Labeled cells were found occasionally in laminae I and V-VII; labeling of contralateral neurons was infrequent. Clarke's column cells were labeled in one animal; however, the C_1 injection was lateral to the LCN and many severed fibers had taken up HRP. In the lower medulla, labeled cells were found ipsilaterally in laminae I and IV of the spinal trigeminal nucleus (pars caudalis), in the ventral portions of the dorsal column nuclei, and in the reticular region underlying these nuclei, predominantly in the immediate vicinity of the obex. These anatomic results concur with published electrophysiological recordings from the LCN in that they support the exclusion of Clarke's column and the inclusion of n. proprius at all spinal levels as projection sources, and, further, demonstrate descending input to LCN from lower medullary neurons, suggesting strong functional interaction between the spino-cervico-thalamic and the dorsal-medial lemniscal systems. (Supported by PHS grants NS 07505 and 5-T01-DE00090.)

1336 CLINICAL EVALUATION OF THE VEGETATIVE STATE USING BILATERAL CORTICAL EVOKED POTENTIALS

J.C. de la Torre and John L. Trimble, Dept. Neurosurg. and Ophthalmology, Univ. Chicago Sch. Med., Chic., Il. 60637

The clinical assessment and prognostic course of severe brain trauma, particularly those resulting in coma, is often difficult and uncertain. The use of cerebral blood flow techniques, EEG, EMI scan, intracranial pressure recording, clinical chemistry and gross neurologic examination provide information that may reflect the extent and locus of the damage but the tests do not generally provide a reliable index on the possible recovery of function from vegetative state.

Male and female subjects ranging in age from 18 to 66 were tested for cortical evoked potentials (CEP) following a diagnosis of coma secondary to acute brain trauma. The patients were tested during mechanical or manual ventilation. Scalp cup electrodes were placed 7 cm from the midline over the right and left somatosensory cortex. A reference electrode was placed on the midline external occipital protuberance with a ground electrode attached to both ear lobes. The scalp recording was differentially amplified by 10^5 using a Grass P511E AC-coupled preamplifier with a passband of 0.3 Hz to 100 Hz (1/2 amplitude points). CEP were obtained by averaging 128 responses to pulsatile current using a Fabri-Tek signal averager. Monopolar current stimuli were delivered bilaterally to each wrist over the median nerve. The constant current pulses had a duration of 0.1 msec and were delivered at a frequency of 1/second. The magnitude of the stimulus current was adjusted to produce a slight thumb twitch. The averaged CEP were analyzed for the peak amplitude and latency of major components. These results show that absence or alteration of the latency response can reliably indicate whether the patient will remain in coma or recover from the vegetative state.

1337 THE CONTRIBUTION OF SPINOTHALAMIC INPUT TO THE RESPONSE OF NOCICEPTIVE Pf, sPf and CL NEURONS. Willie K. Dong, Hiroshi Ryu* and Irving H. Wagman. Dept. of Animal Physiology, Univ. of California, Davis, CA. 95616.

Degeneration studies have shown that the spinothalamic tract (STT) is the only spinal projection which directly terminates in Pf, sPf and CL. Our physiological findings show that the responses of neurons in these nuclei to cutaneous nerve stimulation are due entirely to input transmitted in STT. Neurons were recorded extracellularly in lightly anesthetized (sodium thiopental) cats. 72% of the neurons were nociceptive and had large and often bilateral receptive fields. Some responded exclusively to noxious peripheral stimulation (and to Aδ and C fiber stimulation only) and others to both noxious stimuli and innocuous brisk taps (and to the entire spectrum of A and C fibers). While continuously monitoring the activity of these thalamic neurons to bilateral sural or superficial radial nerve stimulation, portions of the cervical spinal cord between C_2 and C_4 were selectively lesioned in sequence. Bilateral lesions of the dorsal columns and of the dorsal quadrants of the cord containing the spinocervical tracts did not affect the discharges evoked by stimulating either contra- or ipsilateral cutaneous nerves. When these cuts were followed by an ipsilateral hemisection of the cord, discharges to stimulating either the contra- or ipsilateral nerves were significantly reduced but not abolished. The results strongly suggest that the dorsal column and spinocervical pathways are not involved in transmitting somesthetic information to Pf, sPf and CL and that neurons in these nuclei receive bilateral cutaneous input from both contra- and ipsilateral STT projections. Furthermore each STT conveys information originating from both sides of the body. (Supported by NIH Grant AM 16716 and the California Medical Research Foundation).

1338 PROPERTIES OF CELLS IN CAT GRACILE NUCLEUS FOLLOWING TRANSECTION OF THE DORSAL COLUMNS. Jonathan O. Dostrovsky and Julian Millar*. Anatomy Dept., Univ. Coll. London, London WC1E 6BT, England.

There is anatomical and physiological evidence that there are non-dorsal column spinal inputs to the dorsal column nuclei. This study examines the types of stimuli that excite gracile nucleus neurons after section of the dorsal columns. Experiments were conducted on Dial anesthetized cats using tungsten microelectrodes. Histologically verified complete sections of the dorsal columns were made at C5. The number of neurons responding to peripheral stimulation was much lower than in the intact nucleus with many spontaneously active non-driveable cells and silent regions. 70% of the responses were evoked by pressure stimuli, frequently applied to deep tissues and requiring noxious intensities. 16% of the responses were from low threshold hair bending stimuli, but the responses were weak compared to those seen in the intact cat. The remaining responses were from joint movements and were usually weak and phasic and resulted only from extremes of movement. The phasic pressure responses and those to hair bending usually habituated. Some other abnormal responses were also noted: (1) The spontaneous rate of some neurons could be markedly increased by palpation of the limb although no obvious receptive field could be found. The effect frequently required a few minutes of continuous stimulation to reach a maximal rate and then took many minutes (up to 10 min.) to return to normal firing rate levels. This slow onset and decay of 'windup' was also seen in some cases where there was a definite receptive field to deep pressure on the leg. (2) In some cases apparently non-driveable neurons started responding to deep pressure on the leg following a few minutes of strong pressure palpation of the leg eliciting no response.

1339 BEHAVIORAL MEASURES OF CUTANEOUS SENSITIVITY. D. A. Dreyer, M. Hollins, B. L. Whitsel and E. E. Allen*. Dental Res. Cntr. and Depts. of Physiol. & Psychol., Univ. of North Carolina, Chapel Hill, N. C. 27514.

Effects of stimulus velocity and traverse length on ability to correctly identify direction of brush movement (either distal to proximal or the reverse) across the skin of the forelimb were assessed in normal human subjects. A servomotor moved the brush at any of nine velocities ranging from 0.7 to 250 cm/sec. Velocity and direction of movement were randomized within each block of trials. A forced-choice procedure was used to elicit reports of perceived direction of movement from the subjects, whose view of the apparatus was blocked; headphones eliminated auditory cues. Length of skin contacted by the brush was controlled by positioning plates with different sized apertures on the skin; since the accuracy of responding improved with increasing aperture size, the size at which responses were 75% correct ("critical traverse length") could be used as an index of discriminative ability. The duration (rather than the spatial extent) of brush contact with the skin necessary for 75% correct responding ("critical time") was also calculated. The data indicate that: (a) for each body region a reproducible and systematic relation exists among traverse length, stimulus velocity and capacity to identify stimulus direction; and (b) this relation differs for different body regions. Critical traverse length was found to be minimal at velocities in the neighborhood of 10 cm/sec; critical time decreased in a negatively accelerated way with velocity, leveling off at the higher velocities. By either criterion, discriminative ability was better on the thenar eminence than on the preaxial upper arm. These observations will provide the basis for the design of electrophysiological studies of the neural mechanisms which participate in direction detection. (Supported, in part, by NIH research grant NS10865, RDRCP grant DE02668, and GRS grant RR05333; D.A.D. is the recipient of RCDA DE00011.)

1340 MEDULLARY CONTROL OF SPINAL DORSAL HORN NEURONS. H.L. Fields, C.H. Clanton,* A.I. Basbaum,* S.D. Anderson,* Univ. Calif., San Francisco, CA. 94143.

In a previous communication (Cajal Club, 1976), we described a descending pathway in the dorsolateral funiculus (DLF) of the spinal cord which projects primarily to laminae I, II, and V of the dorsal horn. This pathway arises in the ventromedial medulla [Nucleus Raphe Magnus (NRM) and adjacent reticular formation].

Stimulating electrodes were stereotaxically placed in NRM and medial reticular formation of decerebrate cats. The lumbosacral dorsal horn was explored with a microelectrode. Neurons with high threshold inputs located in laminae I and V were inhibited by trains of 0.2 ms, 100-300 μa pulses. Lamina IV-type neurons with only low threshold inputs were not inhibited. NRM stimulating sites were the most consistently effective. The inhibition was blocked by ipsilateral DLF lesions.

Microelectrode studies were also carried out in neurons in ventromedial medulla. In chloralose anesthetized and decerebrate unanesthetized cats, neurons located in NRM and adjacent sites had extensive somatic receptive fields, some covering the entire body. Some increased their discharge to noxious stimuli, including heat and toothed clip. Some of these neurons could be antidromically activated from cervical DLF.

A majority of units tested with i.v. morphine sulfate, 3 mg/kg, showed a marked increase in discharge frequency, in all cases reversed by 0.2 mg naloxone.

We conclude that ventromedian reticular and raphe neurons participate in medullospinal control of pain transmission neurons. This system can be activated by opiate analgesics, electrical stimulation, or natural somatic stimulation.

1341 EFFECTS OF SIMULATED ACUPUNCTURE STIMULATION ON PULP-DRIVEN THALAMIC UNIT ACTIVITY. R. Wayne Fields, Richard B. Tacke*, Robert P. O'Donnell*, and Bhim Sen Savara*. University of Oregon Health Sciences Center, School of Dentistry, Portland, Oregon 97201.

Thalamic field potentials (e.g., Haugen and Melzack, Anesthesiol. 18: 183, 1957; Sparks, et. al., Anesth. Analg. 54: 189, 1975) and unitary responses (e.g., Shigenaga, et. al., Brain Res. 63: 402, 1973; Woda, et. al., Brain Res. 89: 193, 1975) driven by tooth pulp stimulation (presumably noxious) have been identified in several mammalian species in PO (MGmc), CM-Pf (intralaminar) and VPM. The present data characterizes pulp-driven units in contralateral PO, CM-Pf and VPM (identified stereotaxically) of the lightly anesthetized (Ethrane) cat and response alterations elicited by bilateral simulated stimulation of the Hoku Acupuncture points. Pulp stimuli were three 0.1 ms rectangular pulses at 300 Hz, while Acupuncture stimuli were a continuous train of 0.1 ms rectangular pulses at 50 Hz. Thresholds, latencies, and burst lengths to date have averaged 140 μA, 18.4 ms, and 2.4 spikes for PO units (N=30), 170 μA, 30.1 ms and 2.9 spikes for VPM units (N=31), and 250 μA, 28.2 ms, and 3.1 spikes for CM-Pf units (N=18), respectively. Protocols consisted of a control period (0.5-1.5 hrs) Acupuncture stimulation (0.5 hr), and recovery (0.4-1.0 hr). Acupuncture stimulation did not alter spontaneous activity but induced a marked (typically greater than 50%) but reversible attenuation of stimulus-driven burst activity. The Acupuncture-dependent effects may bear significant consequences regarding perception and other activities considered in terms of spatio-temporal summation presently associated with pain.

1342 RESPONSES IN SPINAL NEURONS TO ELECTRICAL STIMULATION OF THE INFERIOR CARDIAC NERVE AND TO MECHANICAL STIMULATION OF SOMATIC STRUCTURES IN THE CAT. R. D. Foreman. Marine Biomedical Institute and Dept. of Physiol. & Biophysics, Univ. of Tex. Med. Branch, Galveston, TX 77550.

The purpose of this study was to determine whether afferent nerve activity originating from the thoracic viscera and from the thoracic somatic structures converge on the same neurons in the spinal cord. Extracellular recordings were made from spinal neurons in the gray matter of the T_1 to T_3 segments of the spinal cord in cats anesthetized with chloralose. Cells were located either by recording spontaneous activity or by observing cells responding to electrical stimulation of the inferior cardiac nerve. The locations of the microelectrode recording sites were determined by histological reconstruction. Individual cells were examined for responses obtained by applying an electrical stimulus to the inferior cardiac nerve and by applying natural stimuli to somatic structures. Afferent volleys, elicited in the inferior cardiac nerve, were recorded from the thoracic sympathetic chain and their conduction velocities were calculated. Adequate cutaneous stimuli included hair movement, touch, pressure and pinch. Many cells increased their discharge as the intensity of the cutaneous stimulus was increased from light touch to pinch. Some cells responded to mechanical manipulation of muscles. The somatic receptive fields were located over the scapula, on the upper thorax or on the upper part of the forelimb. An important observation in this study was that cells located in the dorsal horn and intermediate gray responded to both cutaneous and visceral inputs. The convergence of this information onto cells in the spinal cord may be the underlying neural mechanism related to referred pain involving the thorax and forelimb. Supported by AHA - Texas Affiliate and NIH grant HL 18728.

1343 STIMULUS CODING IN FIRST-ORDER AFFERENT NEURONS INNERVATING THE VIBRISSAE OF ALBINO RATS. John M. Gibson, Wally Welker and Georgia M. Shambes, Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Single-unit electrical activity evoked by deflection of single mystacial vibrissae was recorded (with tungsten microelectrodes) from the V^{th} (trigeminal) ganglia in barbiturate-anesthetized albino (Holtzman) rats. Quantitatively controlled deflections of the vibrissae were applied in their "preferred" directions by a probe attached to a linear feedback-controlled displacement generator. Stimulus waveforms included pulses, trapezoids and sinusoids. A LINC (computer) was used for stimulus control and monitoring and for data display and analysis. A broad range of response properties was observed in a population of approximately 50 units. Angular velocity thresholds (measured with ramp deflections) were distributed over a range exceeding three orders of magnitude, with a median value of about 100^{o}/sec. Approximately 1/3 of the units responded to angular velocities of less than 3^{o}/sec, and exhibited properties associated with the slowly-adapting end of the mechanoreceptor spectrum. The remainder of the population had angular velocity thresholds distributed to well above 3000^{o}/sec. Thresholds for angular displacement (measured with pulse deflections) were also distributed throughout a range in excess of three orders of magnitude, having a median value of about 1^{o}. The most sensitive units responded to deflections of the hair shaft of a few hundredths of a degree, placing them in a sensitivity range comparable to the lowest threshold mammalian cutaneous mechanoreceptors previously described. In summary, the units studied exhibited a broad range of response properties which appear to fall along a continuum rather than into a few discrete categories. (This project was supported by NIH grant numbers NS6225 and NS07026.)

1344 CONVERGENT SOMATIC INPUT ONTO NEURONS OF THE VENTROBASAL COMPLEX OF THE CAT. V. Golovchinsky*, L. Kruger, S. Saporta, B.E. Stein and D.W. Young*. Depts. Anatomy, Anesthesiology and Brain Res. Inst., UCLA Sch. Med., Los Angeles, CA 90024.

Experiments were performed on cats with chronically implanted chambers. The responses of cells in the ventrobasal complex (VB) were classified according to their dynamic properties and the stimulus modality, and the receptive field (RF) was determined for each neuron. The majority of these neurons responding to stimulation of skin and hair showed properties typical of those described in VB by several authors, with small receptive fields located within one segmental dermatome, arranged in topographical order. A second group of cells responded to excitation of sensitive mechanoreceptors but possess RF's extending across dermatomal limits, sometimes including an entire limb and part of the trunk. Response latencies differed significantly when different parts of these large RF's were electrically stimulated, independently of the position of the stimulus. A small number of cells responded to low-intensity tactile stimulation over a large, bilateral RF, described by others as wide-field neurons. In addition, a group of cells showed convergence of input from different primary afferent sources within a small, contralateral RF. Several combinations of heterogenous input were observed: from glabrous and hairy skin; from glabrous skin and the oral mucosa; from sinus hairs and surrounding guard (G) hairs. Using quantitative methods, it was possible to establish other types of interaction, e.g., between G_1 hair and slowly adapting skin input or between G_2 hair and cold receptors. These results demonstrate that some cells of VB are subject to complex sensory interaction outside the constraints of somatotopy and specificity which govern the responses of the majority of VB neurons.
(Supported by USPHS grant NS-5685.)

1345 CUES SUPPORTING RECOGNITION OF THE SPATIAL ORIENTATION OF TACTILE STIMULI. William R. Gould* and Charles J. Vierck, Jr. Dept. Neuroscience, Univ. Fla. Col. Med., Gainesville, Fla. 32610.

Previous experiments with human subjects showed that a small increment (5 mm) in the length of a line impressed on the skin is discriminated (Jones and Vierck, Am. J. Psychol. 86: 49, 1973.). To determine whether this level of activity would be obtained with other variations of linear spatiality, line stimuli were applied longitudinally or transversely to the volar forearm in the present study, and the subjects were required to report on the orientation of each application. Discrimination in this case was poor; the threshold line length for orientation detection was 17 mm. When a movement component was present, orientation detection was much improved, with thresholds of 4.3 mm for light (5 gm) stroking with a probe in the longitudinal vs. transverse directions. When the stimulus probe was glued to the skin so that movement produced skin stretch but not successive stimulation of individual receptive fields, thresholds were less than 1 mm, suggesting that type II slowly adapting units may be the primary receptors for tactile direction sensitivity.

1346 QUANTIFICATION OF SENSORY AND AFFECTIVE VERBAL PAIN DESCRIPTORS BY CROSS-MODALITY MATCHING TECHNIQUES. R.H. Gracely*, P.A. McGrath*, and R. Dubner, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD. 20014.

The magnitudes of sensation or affect implied by 15 sensory pain descriptors (i.e. weak, moderate, strong) and by 15 affective pain descriptors (i.e. unpleasant, distressing, intolerable) were quantified by cross-modality matching to handgrip force and time duration measurements and by numerical magnitude estimation. Resultant scales of sensory intensity and affect were reliable between groups (r = .97, .98), within groups (R = .99, .98) and between separate experiments (r = .99, .99). Word scales of an individual were predicted with equal reliability by either a previous word scale from the same individual (r = .96, .89) or from a word scale derived from a group (r = .96, .89). When used to describe noxious electrocutaneous stimuli (1 sec trains of 100 Hz, monophasic, 1 msec squarewave pulses applied to the wrist), sensory verbal pain descriptors and both sensory and affective cross-modality matches produced similar psychophysical power functions (exponents = 1.5 to 1.7) which were different from the verbal affective function (exponent = 2.8). These results suggest that the verbal descriptor method distinguished affect from sensory intensity. The discriminative power of the descriptor method was demonstrated further in an experiment in which the same electrocutaneous stimuli were scaled immediately before and after the intravenous administration of 5 mg diazepam, a minor tranquilizer. Affective descriptor responses were lowered significantly (p = .004) while sensory descriptor responses were unaltered. These experiments indicate that sensory and affective verbal pain descriptors can be scaled reliably and provide a sensitive tool for the evaluation of pain and pain control methods.

1347 EFFECT OF SKIN TEMPERATURE ON VIBROTACTILE THRESHOLDS. Barry G. Green* (SPON: Lawrence E. Marks). John B. Pierce Fndn. Lab., 290 Congress Ave., New Haven, CT 06519.

Detection thresholds for 30- and 250-Hz vibrotactile stimuli were measured at seven skin temperatures between 20° and 42°C. Thresholds for the 250-Hz stimulus varied as a U-shaped function of skin temperature, with a minimum at 37°C. Thresholds for the 30-Hz stimulus were independent of skin temperature. In a second experiment thresholds were measured for 30-, 50-, 80-, 150- and 250-Hz vibration at three skin temperatures (20°, 37° and 42°C). Temperature significantly affected only the 150- and 250-Hz thresholds. The results obtained for 150- and 250-Hz vibration confirmed an earlier study by Weitz (Journal of Experimental Psychology 28: 21, 1941), who found that changes in skin temperature produced U-shaped threshold functions for 100-, 256-, and 900-Hz stimuli. An explanation as to why skin temperature seems to affect only high-frequency stimulation may be provided by the duplex mechanoreceptor hypothesis (Verrillo, Journal of the Acoustical Society of America 35: 1962, 1963; Mountcastle et al., Science 155: 569, 1967). That is, the skin temperatures tested in the present experiment may have affected only those mechanoreceptive afferents that are primarily sensitive to high-frequency vibration. Experiments are continuing to determine if the effect is actually neural in nature, or whether it may be due to temperature-related changes in the physical properties of the tissue surrounding the mechanoreceptors.

1348 RESPONSES OF CAT KNEE JOINT AFFERENT NEURONS TO ACTIVE CONTRACTIONS OF MUSCLES ACTING AT THE KNEE JOINT. Peter Grigg and Stephen R. Gorfine*. Univ. of Massachusetts Medical School, Worcester, Massachusetts 01605.

Seventy-two afferents from cat medial articular nerve (MAN) were recorded in dorsal root filaments. Responses were observed in relation to a) passive manipulations of the knee, and b) active contractions of quadriceps, semimembranosus (SM) and gastrocnemius muscles. Passive properties were similar to those reported elsewhere; afferents discharged only in response to movements to extreme angular displacements. The largest category of neurons, 36%, could not be activated with any passive or active mechanical stimulation of the knee. Among those neurons activated with passive mechanical stimuli, 63% could also be activated by contraction of quadriceps muscles, and 64% by activation of SM. With muscular contraction, many neurons could be made to discharge in the absence of changes in joint angle. Furthermore, many neurons could be made to discharge at angles intermediate between the limits of movement of the knee, angles at which they were otherwise silent. The magnitudes of these actively-evoked responses were often quite small, usually being a low frequency discharge or a low-frequency burst which adapted rapidly. Thresholds for effects of active contractions ranged from 200 to 2,000 g·cm.

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1349 ON THE SERIAL DEPENDENCY BETWEEN INTERSPIKE INTERVALS IN THE DISCHARGE OF A NEURONAL MODEL. R.J. Harvey (SPON: R. Porter). Dept. Physiol., Monash Univ., Australia & Bristol Univ., England.

When a neurone displays an afterhyperpolarization (AHP) following an action potential, and the AHP accumulates from one interval to another, it is well known that in the output discharge of the cell a short interval between action potentials is likely to be followed by a long one. This gives a negative first order serial correlation coefficient between the intervals in the cell's discharge. In a recent computer simulation study of a neuronal model with a large number of equipotent presynaptic input lines, it was found that cumulative AHP would generate a serial dependency as negative as -0.35 (Harvey, submitted for publication). The output firing was markedly more regular when the firing on each input line was regular rather than random (i.e. Poisson distributed), but the pattern of input firing had very little effect either on the rate or on the serial dependency of the discharge. However, when the model was studied with a relatively small number (< 20) of input lines, the input pattern had an effect both on the regularity and on the serial dependency of the output process. When the discharge on the input lines was random, the serial dependency was dominated by the AHP, but with regular firing on the inputs, large serial dependencies were often observed. The dependency could be either negative or positive and its value depended critically on the relative rates of firing on the different input lines. This could be of considerable relevance in the discharge of, for example, Clarke's column neurones.

1350 ANALGESIC EFFECTS OF CERTAIN NOXIOUS AND STRESSFUL MANIPULATIONS IN THE RAT. R.L. Hayes*, G.J. Bennett*, P. Newlon*, D.J. Mayer, Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD. 20014 and Virginia Commonwealth Univ., Richmond, VA. 23298.

Rats exposed either to electric grid shock (0.35-1.4 ma for 10-20 sec) or to 5 min of rotation (ca. 7.0 transverse g's) were analgesic as measured by tail-flick, hot plate and responses to noxious pressure. Exposure to ether or lateral shaking, both of which have been reported to increase ACTH secretion (a commonly used indicator of stress), did not produce analgesia. Analgesia produced both by shock (SA) and by rotation (RA) persisted for as long as 30 min after exposure to these manipulations. The degree and duration of SA was positively related to the intensity and duration of grid shock. When analgesia was present, normal locomotion and the presence of righting and startle reflexes was observed. Animals also oriented to light tactile, visual and auditory stimuli. Thus neither SA nor RA resulted from generalized sensory, attentional or motoric deficits. Studies employing classical conditioning procedures showed that 2 daily exposures to grid shock resulted in significant increases in tail-flick latencies when animals were placed on the grid but not shocked on the third day. Neither naloxone, (1 mg/kg), a narcotic antagonist, nor Librium (5 mg/kg), a minor tranquilizer, antagonized SA measured after shock or application of conditioning procedures. However, SA was abolished by spinal cord transection (T_2-T_3). These results suggest: (1) the presence of a supraspinal, non-narcotic system selectively modulating nociceptive input and conditionable by Pavlovian contingencies; (2) that diverse manipulations result in analgesia mediated by a supraspinal system or systems that may be physiologically distinct from those involved in analgesia produced by the administration of narcotics or brain stimulation. Supported in part by NIDA grants 00576, 00490 and 00296.

1351 THE LOCATIONS OF ASCENDING TRIGEMINAL PROJECTION NEURONS WITHIN AND ADJACENT TO TRIGEMINAL NUCLEUS CAUDALIS IN THE CAT. Susan Hockfield and Stephen Gobel. Neurobiology and Anesthesiology Branch, NIDR, NIH, Beth., MD. 20014; and Dept. Anat., Georgetown Univ., Wash., DC. 20007.

The locations of trigeminal projection neurons in nucleus caudalis (CAUD) were determined by utilizing the method of retrograde axonal transport of horseradish peroxidase (HRP). In an attempt to unilaterally fill the thalamus with HRP, multiple injections (8-13 sites) were made stereotaxically (0.5 µl of 33% HRP/site). In one animal, HRP completely filled the thalamus unilaterally while in a second animal the HRP filled only the caudal two-thirds. In both cases the HRP extended into the contiguous midbrain tegmentum. Camera lucida drawings were made of 50 µm serial sections which extended through the entire length of the contralateral CAUD and the C1 segment of the spinal cord. They illustrate the precise locations, sizes, and shapes of the labeled neurons. The marginal layer contains labeled neurons 7-20 µm in diameter. Virtually all of these are found in the outer half of this layer between the spinal V tract and the medial border of the underlying row of deep bundles. Labeled marginal neurons are most numerous in the most rostral millimeter of CAUD and diminish in number in the succeeding three millimeters. Outpocketings of the marginal layer into the spinal V tract (interstitial nucleus of Cajal) also contain labeled cells. The medial half of the magnocellular layer contains labeled neurons and almost all of these are in the caudal half of CAUD. Numerous labeled neurons occur in clusters along and subjacent to the medial border of CAUD. The most ventral of these are continuous with the labeled neurons of the lateral cervical nucleus of the C1 segment of the spinal cord and may represent a trigeminal extension of the lateral cervical nucleus.

1352 PAIN RELIEF BY ELECTRICAL STIMULATION OF THE CENTRAL GRAY MATTER IN HUMANS. Yoshio Hosobuchi*, John E. Adams* and Rita Linchitz.* (SPON: Nancy M. Lee) Dept. Neurosurg., Sch. Med., UCSF, San Francisco, CA. 94143 .

Relief of intractable pain was produced in six human patients by stimulation of electrodes chronically implanted in the periventricular and periaqueductal gray matter. The level of stimulation sufficient to induce pain relief seems not to alter the acute pain threshold. Indiscriminate repetitive stimulation produced tolerance to both stimulation-produced pain relief and the analgesic action of narcotic medication; this process could be reversed by abstinence from stimulation. Stimulation-produced pain relief was reversed by naloxone in 5 out of 6 patients. These results suggest that satisfactory alleviation of chronic pain states in humans may be obtained by electronic stimulation alone.

1353 UNMYELINATED SENSORY FIBERS IN THE CAT L7 AND S1 VENTRAL ROOTS. H. Ito* and R.E. Coggeshall. Depts. of Anatomy and Physiology and The Marine Biomedical Institute, Univ. of Texas Medical Br., Galveston, Texas 77550.

In a previous study, it was shown that approximately 15% of the axons in the S3 and Cal ventral roots of the cat were unmyelinated and arose from dorsal root ganglion cells. Unmyelinated axons attached to peripheral receptive fields could be demonstrated in these same roots, and two-thirds of the demonstrated receptive fields were located in the viscera. By contrast, the cat L7 and S1 ventral roots contain an even higher proportion of unmyelinated fibers that arise from dorsal root ganglion cells. The present report describes 52 unmyelinated fiber receptive fields in the L7 and S1 cat ventral roots. In these roots, two-thirds of the receptive fields are somatic and the remainder are visceral. The majority of the somatic sensory fibers are mechanical nociceptors, polymodal nociceptors or deep pressure receptors, but there are six thermal receptors and two low threshold mechanoreceptors in these roots. The visceral fields were located in the rectum or bladder and were either nociceptive or responded to distension. It is concluded that the L7 and S1 cat ventral roots contain a significant number of sensory fibers. The majority of the sensory fibers are somatic nociceptors, but other fiber types can also be found. Supported by NIH grant NS 11255.

1354 COLD RECEPTOR TRANSDUCTION MECHANISMS IN THE MONKEY. Kenneth O. Johnson, Ian Darian-Smith and Alan W. Freeman*. Dept. Physiol. Univ. Melbourne, Victoria, Australia.

When the hairless skin is cooled rapidly an A-delta cold fiber responds with a high discharge rate which decays within a few seconds to a steady rate (Darian-Smith et al., J. Neurophysiol. 36:325-370, 1973). A growing depression of the fibers' responsiveness to succeeding cooling stimuli parallels the decay in discharge rate. When the skin temperature is returned to normal, the responsiveness of the cold fiber returns to normal along a near-exponential time course which takes from 10 to 50 seconds depending on the severity of the depression. The time constant of depression decreases systematically with increasing stimulus intensity, from 5-10 sec. for mild cooling (less than 1C) to 2 sec. for moderate cooling (8C). The recovery time constant depends only on the level of depression at the end of the cooling stimulus and not on the cooling amplitude or duration that produced it. Cold receptors recover from mild depression (less than 50%) within 10 sec. (2 x time constant) but require 50 sec. or longer (2 x time constants) to recover from more severe depression (75%).

Transduction models with various chemical kinetic properties were investigated. All of the data are consistent with a transduction model with an autocatalytic recovery process.

1355 STEADY-STATE AND DYNAMIC RESPONSES OF PRIMATE WARM UNITS TO WARMING. Dan R. Kenshalo and Roland Duclaux*. Florida State University, Tallahassee, Fla. 32306

Specifically sensitive warm units (warm receptors and their innervating primary axons) show steady-state responses to static temperatures between approximately 30° and 48°C. As reported by Hensel and Iggo (Pflügers Arch., 1971, 329, 1-8) there appear to be two types of warm units in primate skin. Some show a rapid increase in the steady-state response from the 30°C adapting temperature (AT) to a peak of between 15 to 20 impulses/sec at ATs of 45° to 48°C. A further small increase in the AT (1°C) results in silence. The effect can be reproduced many times on the same unit. The majority of warm units show a slow increase in the steady-state response from approximately a 30°C AT to a mean peak of frequency of approximately 5 impulses/sec at approximately a 43°C after it decreases to near zero at the 49°C AT.

Dynamic responses to increases in skin temperature (+ΔT) show a phasic increase in frequency that varies in magnitude with the AT, rate and intensity of warming. Warm units are most sensitive to +ΔTs from the 40°AT, curvilinear stimulus-response functions are observed, and the faster rate produces larger responses. In agreement with the frequent observations that warm sensations have a longer latency of onset and slower build-up to a peak intensity than cold sensations, warm units exhibit a longer response latency and slower increase in frequency to peak than do cold units.

(Supported by USPHS Grant NS02992.)

1356 THE CONSEQUENCE OF NEONATAL VIBRISSAE REMOVAL ON TRIGEMINAL PATHWAYS IN THE RAT. I. DEVELOPMENT OF NORMAL AND ANOMALOUS THALAMOCORTICAL PROJECTIONS (TERTIARY AFFERENTS). Herbert P. Killackey and Gary Belford*. Department of Psychobiology, University of California, Irvine, CA 92717.

We have previously demonstrated that removal of a row of vibrissae at birth results in an anomalous organization of the thalamocortical projections associated with that row of vibrissae in the adult rat (Brain Res., 1976, 104, 309-315). This anomalous organization could arise in one of two distinct ways. The projections could form normally and then undergo a subsequent reorganization or the initial aggregation of the system may be anomalous.

The present experiment was designed to determine which of these alternatives is correct. Normal and experimental (vibrissae damage on day 0) animals were sacrificed on day 0,1,2,3,4,5 and 6. The brains were cut in a cryostat in a plane tangential to somatosensory cortex and the sections processed with succinic dehydrogenase histochemistry.

On day 3 the discrete organization of the thalamocortical projections is first discernible. By day 5 the pattern of projections is essentially the same as that seen in the normal adult. The development of the anomalous projections follows a similar time course. On day 3, when faint segmentation is first observable, it is impossible to differentiate between normal and anamolous projections. However, by day 5 the anomalous pattern is clearly established.

These results suggest that after vibrissae damage the anomalous pattern is formed in the initial development of the system and not as a later reorganization. Further, the results suggest that a peripheral structure plays an important organizational role in the development of a central neuronal pathway. Finally, it is important to note that this effect occurs with extreme rapidity (a maximum of five days). SUPPORTED BY NSF GRANT #GB 41294.

1357 SELECTIVE REVERSAL BY NALOXONE OF PERIAQUEDUCTAL GRAY MATTER (PGM)-INDUCED INHIBITION OF REFLEX AND NEURONAL RESPONSES TO TOOTH PULP STIMULI. G.E. Lucier*, R. Dubner and B.J. Sessle, Fac. Dent., Univ. Toronto, Canada

PGM stimulation is reported to exert powerful analgesic effects, and we recently showed that it can suppress the jaw-opening reflex (JOR) and responses of neurones in trigeminal (V) brain stem nuclei oralis and caudalis to noxious stimuli (Sessle et al., Can. J. Physiol. Pharmacol.54, 66, 1976). This study was extended in anaesthetized cats to test effects of naloxone (reportedly a specific opiate antagonist) on PGM-induced inhibition of oralis cells responsive to tactile and/or tooth pulp (TP) stimuli. Oralis is a site of V cells relaying to higher perceptual levels and of interneurones involved in JOR initiation. Bipolar stimulation (100 Hz, 200 msec train of 0.2 msec pulses) of PGM suppressed the JOR evoked by TP stimuli, at lower intensities (0.1-0.4 mA) than its inhibitory effect on the JOR evoked by low-intensity infraorbital nerve (IO) stimuli. Naloxone (0.4 mg/kg i.v.) had no apparent effect on JOR or on the inhibition induced by PGM (or somatosensory cortex) stimulation on the IO-evoked JOR but did raise the PGM stimulus intensity needed for suppression of the TP-evoked JOR. This selective reversal of PGM inhibition by naloxone was also noted in V-thalamic relay cells and non-relay cells in oralis. PGM conditioning suppressed TP responses in 54/55 oralis cells studied; naloxone reversed this effect in all 8 cells tested. In contrast, PGM stimulation inhibited responses to low-intensity IO or tactile stimuli in only 14/29 oralis cells, and naloxone did not reverse this suppression in 8/10 cells tested. The findings suggest that PGM stimulation may activate descending paths that can selectively inhibit different types of V cells. The naloxone-reversed inhibition of TP-evoked responses may be related to a descending path to oralis that is also activated by narcotic analgesics. (Supported by Canadian M.R.C.)

1358 PROJECTIONS OF THE LATERAL CERVICAL NUCLEUS AND THE DORSAL COLUMN NUCLEI TO REGIONS ADJACENT TO AND WITHIN THE N. VENTRALIS POSTEROLATERALIS OF THE CAT THALAMUS. D. C. Mash*, I. G. Worden*, and K. J. Berkley (SPON: Judith Tunkl). Dept. Psychol., Fla. St. Univ., Tallahassee, FL. 32306.

The projections of the lateral cervical nucleus (LCN) and the dorsal column nuclei (DCN) to the cat thalamus were studied using a differential labeling strategy in which, in the same cat, one of these pathways was labeled using autoradiographic tracing methods and the other was labeled using degeneration tracing methods. The results show that there is considerable overlap between the LCN and DCN terminations within n. ventralis posterolateralis (VPL). Compared to the relatively homogeneous distribution of DCN preterminals and terminals within the VPL, the LCN preterminals and terminals tend to be distributed in "bursts" and to be most concentrated at the edges of its VPL projection region, thereby forming a discontinuous shell surrounding most of VPL. In addition, at the more rostral levels of VPL, the LCN projections extend beyond the lateral, dorsal and rostral borders of the DCN projections into a region that also appears to receive input from the cerebellum, the spinothalamic tract and the n. Z. When considered together with the results of others, these results suggest that fibers from LCN that project to the thalamus may contribute to the proprioceptive response characteristics of cells within the regions at the borders of VPL as well as to the cutaneous properties of cells within VPL.
(Supported by PHS grants 1K04-NS00118 and 5R01-NS11892 from the National Institutes of Health.)

1359 THE EFFECT OF STIMULATING TRIGEMINAL AFFERENTS ON THE RESPONSE OF SPINOTHALAMIC NEURONS TO MECHANICAL STIMULI. D. McCreery* and J.R. Bloedel. Depts. Neurosurg. and Physiol., U. of Minn., Minneapolis, Mn. 55455

In experiments designed to evaluate interactions between the trigeminal and spinothalamic systems, the effect of electrically stimulating the infraorbital branch of the trigeminal nerve on the response of lumbosacral spinothalamic neurons to mechanical stimuli was examined in cats anesthetized with alpha chloralose. Stimulation of the nerve produced a depression of the responses of these cells to only certain types of mechanical stimuli. The greatest effect was on the wide dynamic range response to sustained and intense mechanical stimuli, while the phasic response to light or transient mechanical stimuli was relatively unaffected. This selectivity was observed even for those spinothalamic neurons which exhibited both a wide dynamic range response as well as a phasic response to the appropriate mechanical stimuli. The reduction in the response of spinothalamic neurons occurred when the strength of the electrical stimulus was 2 times that required to activate the alpha group of trigeminal afferents. The latency of this reduction indicates that the descending pathway mediating this effect has a conduction velocity of at least 10m/sec. The selective reduction of the wide dynamic range response suggests that this descending pathway exerts its action either on interneurons projecting to spinothalamic neurons or on terminals of the primary afferent fibers which mediate the wide dynamic range response. This work was supported by a research grant from Medtronic, Inc., and NIH Grants R01-NS09447 and R01-NS13002.

1360 CHANGES IN PRIMATE PACINIAN CORPUSCLES FOLLOWING VOLAR PAD EXCISION AND SKIN GRAFTING. Stephen H. Miller, M.D., Irena Rusenas, B.S.*, and William P. Graham, III, M.D. Dept. Surgery, Div. Plastic Surgery, Pennsylvania State University, Hershey, PA 17033.

Repair of skin and soft tissue defects following fingertip avulsions can be achieved by a variety of methods including split-thickness skin grafts, or the avulsed skin may be defatted and replaced as a free full-thickness skin graft. Histologic studies to document reinnervation and the fate of terminal sensory receptors following avulsion injury and repair by skin grafts in primates are lacking.

Human fingertip avulsion injuries were simulated by excising volar digital pads in stump-tailed monkeys. Half the defects were covered with split-skin grafts from the forearm and half with full-thickness grafts of fingertip skin. Innervated pacinian corpuscles were found in the center of these grafts 3 months after the operation. The site of origin of these corpuscles is undefined.

Denervation and devascularization of pacinian corpuscles resulted in alterations of their gross architecture, size, and innervation. The possibility exists that these alterations result from a dynamic adaptation of pacinian corpuscles to environmental stress.

1361 THE EFFECT OF COOLING RATE ON THE DYNAMIC RESPONSE OF CAT COLD FIBERS. H.H. Molinari and D.R. Kenshalo. Dept. Psychol., Fla. St. Univ., Tallahassee, Fla., 32306

Cold fibers innervating the cat's face were studied by extracellular recording in the trigeminal ganglion. The responses of the units to 2.5°C cooling at six rates (0.04°, 0.06°, 0.1°, 0.5°, 1°, and 2°C/sec) from three adapting temperatures (20°, 30°, and 40°C) were examined. The average frequency during a period including the temperature change and the first 3 sec at the lower temperature was used as an index of the dynamic response of the individual fibers. The average dynamic response for the population, calculated by averaging these values across the fibers, was then compared to human psychophysical data. As the cooling rate was increased from 0.5° to 2°C/sec, the average dynamic response did not vary significantly. However, for cooling rates below 0.1°C/sec the dynamic response was greatly reduced. Human subjects estimate the intensity of the thermal sensations produced by comparable stimuli to be constant across cooling rates of 0.5° to 2°C/sec. Human thermal thresholds are constant with cooling rates above 0.1°C/sec but increase with decreases in stimulus rate below this value. These results indicate that the average dynamic response correlates well with the psychophysical data on sensation magnitude. (Supported by NSF Grant GB-30610.)

1362 RESPONSE OF NEURONS IN SPINAL TRIGEMINAL NUCLEUS TO CUTANEOUS THERMAL STIMULATION. James T. Molt and Dennis A. Poulos. Div. Neurosurg. and Dept. Physiology, Albany Medical College, Albany, N.Y. 12208

Neurons located within the spinal trigeminal nucleus which were thermally sensitive but insensitive to mechanical stimulation of their peripheral receptive fields were studied in cats anesthetized with nembutal or urethane. The response of single units to constant temperatures and to rapid temperature changes were recorded extracellularly with microelectrodes. Units with fields within the third division (lip and tongue) and units with fields within the second division (hairy skin) showed responses consisting of 1) ongoing firing rates at a skin temperature of 35°C 2) phasic rate increases to temperature changes below 35°C 3) phasic rate decreases to temperature changes above 35°C and 4) temperature dependent activity over a wide range of temperatures held constant. Some units were observed to receive convergent inputs from fields as disparately located as the lip and tongue. Units with receptive fields limited to the glabrous nasal skin displayed thermal response characteristics that differed from the above. One population showed 1) ongoing activity over a range of constant skin temperature 2) minimal phasic rate increases to cooling below 35°C and 3) prominent phasic decreases to warming above 35°C. A second population showed little or no ongoing activity to constant temperatures but did display prominent phasic rate increases to cooling below 35°C. These results indicate that processing of thermal information can occur at the first synapse in the trigeminal afferent pathway. Convergence of specific thermoreceptors has been observed and in addition, a population with receptive fields confined to the glabrous nose appears to be extracting only certain features of their primary afferent input. Supported by NIH grant NS11384.

1363 WHITE NOISE ANALYSIS OF THE DYNAMIC SENSITIVITY OF MUSCLE RECEPTOR AFFERENTS. G.P. Moore, J.A. Boles*, R.M. Reinking*, and D.G. Stuart. Dept. of Biomedical Engr., Sch. of Engr., Univ. So. Calif., Los Angeles, CA 90007, and Dept. of Physiology, Sch. of Med., U. Arizona, Tucson, AZ 85724.

Previous studies of the dynamic response characteristics of muscle receptor afferents have utilized step, ramp, or sinusoidal changes in muscle length. We studied the passive responses of type Ia, Ib, and spindle group II afferents from the medial gastrocnemius muscle of anaesthetized cats. The muscle was connected by its tendon to a servo-puller which changed the length of the muscle according to a Gaussian white noise command signal. Each receptor was studied at several different operating lengths, and with different RMS values of perturbation (ranging from 50 to 500 microns). Bandwidths were usually 200Hz or below. Muscle length and force at the tendon were recorded simultaneously with the spike train of the afferent. By averaging the length sequences preceding each receptor spike it is possible to determine the differences in spike-evoking length trajectories associated with each receptor type, and how these change as functions of muscle length and noise amplitude. By comparing the static discharge of each receptor with the discharge pattern evoked by low amplitude random length changes, new measures of dynamic length sensitivity and frequency response have been obtained which are more sensitive and reproducible than previous estimates, and can be obtained from short experimental runs. (Supported by NIH Grant NS 11298, NS 07888, and FR 05754.)

1364 THE EFFECT OF DORSOLATERAL SPINAL CORD(DLF) LESIONS ON ANALGESIA FROM MORPHINE MICROINJECTED INTO THE PERIAQUEDUCTAL GRAY MATTER(PAG) OF THE RAT. R.Murfin*G.J.Bennett* and D.J.Mayer, Depts. of Psychology and Physiology, Va.Commonwealth U., Richmond, Va. 23298. (SPON: S.J. Goldberg)

Much recent evidence has converged to suggest that a pain inhibitory system exists in the central nervous system. Morphine analgesia(MA)appears to result, at least in part, from action of the drug on a medial brainstem component of this system with an ultimate inhibitory action on transmission of nociceptive information through the spinal cord. It is of considerable interest to identify the descending spinal pathways involved in MA from supraspinal action of the drug. Analgesia from electrical stimulation of the PAG is abolished by a DLF lesion(Basbaum et.al., Proc. IASP,1975). When morphine is injected i.p., bilateral DLF lesions reduce, but do not abolish, MA on the tail flick(TF) test in the rat(Hayes et.al., Proc.Soc.Neurosci.,1976). The present study investigated the effect of DLF lesions on MA when the drug is limited to supraspinal sites by microinjection into the PAG. One to two weeks after implantation of microinjection cannulae MA(8μg.,0.5μl.) was assayed by the TF test. One day after testing, animals received either a T3 level bilateral DLF lesion (n=8), bilateral dorsal column(DC) lesion(n=5), or control surgery(n=10). Nine days later MA was again assayed. MA was abolished in all animals with DLF lesions(mean MA reduced from 80% to -9%). MA in control and DC lesioned animals was unchanged. Preliminary evidence indicates that MA from PAG microinjection as assayed by hot plate and pinch tests is reduced but not abolished by DLF lesions. These results suggest that inhibition of the spinal TF reflex by morphine acting at supraspinal sites is mediated by descending fibres in the DLF.(Supported by PHS grant DA 00576)

1365 RESPONSE VARIATIONS OF NEURONS IN ROSTRAL AND CAUDAL PORTIONS OF THE SENSORY TRIGEMINAL COMPLEX FOLLOWING TOOTH PULP STIMULATION. Samuel G. Nord. Dept. Neurol., Upstate Med. Ctr., SUNY, Syracuse, N.Y. 13210.

Although neurons which receive projections from the canine tooth pulp have been identified electrophysiologically in each of the sensory nuclei of the brain stem trigeminal complex, they are not distributed uniformly. Rather, a relatively dense cluster of the neurons lies rostrally in a nucleus principalis-subnucleus oralis region while a similar, though smaller, cluster is situated caudally at the level of the obex in the subnucleus caudalis. Functional differences between the two groups of neurons are suggested by the published data, but variations in experimental procedures have precluded systematic, quantative comparisons. The present investigation addresses this problem. Using immobilized, lightly anesthetized cats, the responses of neurons located in each of the two regions were studied following electrical stimulation of the canine tooth pulp through embedded, bipolar electrodes. These procedures activated 122 neurons in the rostral region and 45 in the caudal one. Although some similarities were noted, the two populations tended to display different, though overlapping, distributions of response characteristics. For example, "rostral" cells generally had lower thresholds and briefer response latencies; they frequently responded with more spikes to maximally effective stimulus values; they usually had more restricted non-dental fields and they responded to contralateral pulpal stimulation less often. Moreover, the responses of rostral neurons were less susceptible to suppression by the application of prior conditioning stimuli to either ipsilateral or contralateral pulpal sites. Supported by NIH Grant NS10814.

1366 Localization of Specific Thermoreceptors in Spinal Trigeminal Nucleus of the Cat. Dennis A. Poulos, James T. Molt and Kevin D. Barron, Div. Neurosurgery and Depts. Physiology and Neurology, Albany Medical College, Albany, N.Y. 12208.

There exists little information on the organization of specific thermoreceptive afferents within the spinal trigeminal nucleus caudalis, a region that when interrupted in man, is known to produce analgesia and thermanesthesia. Extracellular recordings were obtained from neurons located within the spinal V nucleus caudalis (approximate level of the obex) in cats anesthetized with nembutal or urethane. Neurons responsive to cutaneous thermal changes (within the range of 15-45°C) were identified and small electrolytic lesions (50-100 μ) were placed within the recording sites. Cells identified as thermoreceptors were insensitive to mechanical forms of stimulation and demonstrated all of the thermal response properties of specific cold receptors. Measurements of recording depths and the histological locations of microlesions showed thermoreceptors to be clustered in the outer marginal zone of the trigeminal nucleus caudalis. While a search for nociceptive afferents was not made in this study, neurons that responded only to warming above 45-47°C or the application of ice water immediately following a warming stimulus were found intermingled with specific thermoreceptors. The thermal "nociceptors" were primarily associated with tooth receptors. A precise somatotopy appeared to be maintained in that thermoreceptive afferents were always found to be located in a position superficial to cells responsive to light tactile stimulation of the same peripheral receptive fields. Supported by NIH grant NS 11384.

1367 EFFECTS OF DORSOLATERAL SPINAL CORD LESIONS ON NARCOTIC AND NON-NARCOTIC ANALGESIA IN THE RAT. D.D. Price, R.L. Hayes*, G.J. Bennett*, G.L. Wilcox, and D.J. Mayer, Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD. 20014 and Depts. Psychol. & Physiol., MCV, Richmond, VA. 23298.

These studies examined the effects of bilateral lesions of the dorsolateral funiculus (DLF) of the rat spinal cord (T_2-T_3) on analgesia produced by a systemic injection of 4 mg/kg of morphine or by a 20 sec exposure to 1.0 mA of transcutaneous electric shock, termed shock analgesia (SA). Analgesia was measured by a modified version of the D' Amour-Smith tail-flick test. The term analgesia as used here denotes only the observation of reliable increases in latencies of nociceptive reflexes. Lesions which included only the DLF reduced morphine analgesia (MA) by 71 percent, but had no effect on SA observed in the same rats. Baseline tail-flick latencies were not changed by DLF lesions. Lesions which included both the dorsal columns and DLF did not effect SA and produced no greater reduction in MA than lesions of the DLF alone. Control lesions restricted to the dorsal columns had no effect on SA or MA. In view of previous work indicating that both MA and SA result, at least in part, from activation of supraspinal structures, these data further indicate that (1) shock-produced analgesia and narcotic analgesia are mediated by separate descending spinal pathways, and (2) the supraspinal expression of narcotic analgesia requires the integrity of pathways descending in the DLF. The observation that DLF lesions block both brain stimulation-produced analgesia (Basbaum et. al., First World Congress on Pain, 1975) and morphine analgesia provides further evidence that both forms of analgesia are subserved in part by common neural mechanisms (supported in part by PHS grant DA 00576 to DJM).

1368 THE SECOND SOMATIC SENSORY AREA (SmII) OF OPOSSUM NEOCORTEX. Benjamin H. Pubols Jr. Dept. of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA.

Organization of the neocortical second somatic sensory receiving area (SmII) has been examined in anesthetized Virginia opossums. Microelectrodes have been used to record the activity of cortical neurons in response to mechanical stimulation of the body surface. SmII occupies a surface area of 2.5-3.5 mm^2, and is situated between SmI medially, and the rhinal fissure laterally. The trigeminal representation is located anteromedially in SmII, the hindlimb representation posterolaterally, with the forelimb representation in between. Approximately 48% of SmII is devoted to representation of the head and face, 38% to the forelimb, and 14% to the remaining body surfaces, figures comparable to those of SmI when the "lateral trigeminal area" is excluded (Pubols, Pubols, DiPette, & Sheely, J. comp. Neurol., 1976, 165, 229-246). Approximately 75% of all SmII head/face peripheral receptive fields (RF's) were bilateral, while 25% of forelimb RF's, and 100% of hindlimb RF's were. For a given cutaneous surface, SmII RF's are larger than those for SmI. Of the bilateral RF's, approximately 15% were asymmetrical, in that either the ipsilateral component of the RF occupied less cutaneous surface area than the contralateral component, or more intense stimulation of the ipsilateral component was required to yield a cortical response. No strictly ipsilateral RF's have been identified. Activating peripheral stimuli varied from deep pressure to as little as 5 gm/mm^2 pressure (von Frey stimulus). SmII is contained entirely within an area yielding evoked potentials responses to auditory click stimuli, and there was no evidence for a separation into modality-place specific vs. modality-place nonspecific regions, as has been found in some placental mammals.

1369 CHEMICAL SENSITIVITY OF CAT DORSAL HORN NEURONES ACTIVATED BY NOXIOUS STIMULI. Mirjana Randic and Vjekoslav Miletic*. Iowa State University, Ames, IA 50011

Using the microelectrophoretic method in conjunction with extracellular recording and dye-marking of cells we have studied the chemical sensitivity of the cat dorsal horn neurones (particularly at the level of Rexed's Laminae I and II) selectively activated either by noxious mechanical and/or thermal stimulation or by a volley in Aδ or C fibres to acetylcholine, nicotinic and muscarinic agents and substance P.

We have found that a majority of the examined neurones were excited by acetylcholine. Only occasionally depression of the evoked firing was observed. Characteristics of the receptors mediating excitant effects of acetylcholine in the dorsal horn neurones selectively activated by noxious stimuli will be discussed.

Substance P caused a prolonged excitation of units activated by noxious stimulation of the skin.

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1370 INTRAORAL REPRESENTATION IN PRIMARY SOMATOSENSORY NEOCORTEX OF THE CAT. Jon L. Richter*, and Lillian M. Pubols. Dept. of Anat., Med. Col. of Penna., Phila., Pa. 19129.

Recent unit cluster mapping studies of intraoral representation in nucleus ventralis posteromedialis of the thalamus (VPM) (Bombardieri, R.A. et al., J. Comp. Neurol., 163:41-64, 1975) have revealed that its organization differs in a number of respects from that of its postcranial counterpart, the nucleus ventralis posterolateralis (VPL). In the mouth region of VPM in several species many ipsilateral and discontinuous receptive fields involving opposing teeth were identified. The present study examines electrophysiologically the projection of this region to the primary somatosensory neocortex (SI).

Cats lightly anesthetized with methoxyflurane were prepared to facilitate access to all mouth parts. The head region of SI was explored stereotaxically with microelectrodes while light pressure was applied to oral and perioral surfaces with hand held probes. Electrode tracts and recording loci were identified by histological reconstruction.

Multi- and single unit responses were recorded from the coronal gyrus and from the depths of the coronal sulcus in response to stimulation of the teeth, gingiva, vestibule, palate, and tongue. Responses to stimulation of ipsilateral and contralateral mouth parts occurred with approximately equal frequency and were organized in a continuous topographic map. Wide bilateral receptive fields usually included teeth. Many single neurons had discontinuous receptive fields involving a pair of opposing teeth or groups of opposing teeth.

Thus some of the unique features of the specific intraoral somatosensory system identified in VPM with unit cluster techniques are also apparent with single unit analysis in primary somatosensory cortex.

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1371 SYMPATHETIC EFFERENT MODULATION OF ACTIVITY IN FROG CUTANEOUS MECHANORECEPTORS. William J. Roberts and Robert B. Jones*. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon 97210.

Sympathetic efferent control of cutaneous mechanoreceptors has been previously studied in frogs using isolated skin-nerve preparations (Lowenstein, J. Physiol. 132:40, 1956) or reflected skin flaps (Chernetski, J. Neurophysiol. 27:493, 1964). The present study is a re-examination of this mechanism for identified mechanoreceptors with the skin in vivo.

Single afferent unit activity was recorded from small filaments of the cutaneous branch of the peroneal nerve in frogs anesthetized with ethyl-m-aminobenzoate. Units were characterized by their responses to repeatable mechanical stimuli. Responses to test stimuli were compared to responses following conditioning electrical stimulation of the sympathetic trunk. As in the studies cited above the responses of some units were potentiated while others were unaffected. Unlike previous reports many units showed decreased responses following sympathetic stimulation. Both types o f sympathetic effects occurred at short latency (about 1 sec) and persisted for several tens of seconds. Afferent units having similar response characteristics to mechanical stimulation were not all similarly affected by sympathetic stimulation. These results indicate that the in vivo sympathetic control of mechanoreceptors likely serves some more complex function than simply the enhancement of peripheral sensory input as previously suggested (Chernetski, J. Neurophysiol. 27:493, 1964).

1372 THE MORPHOLOGY OF COLLATERALS FROM IDENTIFIED CUTANEOUS PRIMARY AFFERENTS. P.K. Rose, A.G. Brown† and P.J. Snow*. Dept. of Vet. Physiol., University of Edinburgh, Scotland, EH9 1QH.

While there is ample evidence that cutaneous primary afferents terminate in the dorsal horn, little is known about the anatomy of identified cutaneous afferents. Terminal arborisations have been found in the dorsal horn of Golgi stained material, but direct evidence that such arbors originate from cutaneous afferents is lacking. In the present experiments on chloralose anaesthetized cats, horseradish peroxidase was injected, by iontophoresis (Snow, et al., Science 191:312, 1976), into single primary afferents at the dorsal root entry zone. Prior to injection the mechanoreceptive properties of the afferents were classified, eg. type T or G hair, type I or II slowly adapting, etc. After histochemical processing, the axons and their collaterals were reconstructed from 50 or 100 μm serial coronal sections.

Usually from 5 to 7 mm of axon were stained and each primary afferent gave rise to several collaterals (average 7; range 4-16). All collaterals except those originating from the most rostral and caudal segments of the stained axon, gave rise to arbors within in the dorsal horn. The arbors of a single primary afferent were usually alike in shape and branching pattern. Collaterals from afferents innervating hair follicles descended into the dorsal horn and, after dropping off several branches, reversed direction and formed a flame-shaped arbor. This pattern of collateral branching was characteristic of hair afferents and was not seen for other cutaneous afferents. These results suggest that primary afferents innervating mechanoreceptors in the hindlimb of the cat give rise to collaterals whose arborisation pattern is related to the receptor and its properties. (Supported by a grant to A.G.B. from the MRC).

1373 PRIMARY AFFERENT CONVERGENCE ON NEURONS IN THE SPINAL TRIGEMINAL NUCLEUS. Howard S. Rosing* and Kenneth V. Anderson. Dept. Anatomy, Emory Univ., Atlanta, Ga. 30322.

Subnucleus caudalis (NVCaud) of the spinal trigeminal system is known from clinical and experimental observations to play an important role in pain perception. Tooth pulp afferent fibers represent a relatively homogenous group of small-diameter axons which project bilaterally to NVCaud. The present experiment was designed to study the convergence of primary afferent fibers from the canine teeth onto the second-order neurons within NVCaud using neurophysiological and computer analysis techniques.

Responses were recorded from 136 neurons in NVCaud from 24 cats, following bipolar, electrical stimulation of the four canine teeth. Neurons were classified according to which canine teeth were effective in eliciting a response. These were (1) a single ipsilateral canine (38%); (2) both upper canines (22%); (3) both lower canines (19%); (4) both ipsilateral canines (18%); (5) both ipsilateral canines and either contralateral canine (2%); and (6) all canine teeth (1%). Conduction velocities ranged from 1.0-25.0 m/sec, indicating that both C-fibers and A-delta-fibers were activated from tooth pulp stimulation. The response patterns of neurons were generally found not to be dependent on the degree of canine afferent convergence. Neurons in most categories displayed excitatory, excitatory-suppressed and purely suppressed responses. Additionally, many neurons (12%) displayed response patterns which were dependent on the site of stimulation. Neurons which responded to several sites tended to have more complicated response patterns than neurons that responded to two or less sites. Some neurophysiological and anatomical mechanisms that might account for the observed data will be discussed.

1374 SENSORIMOTOR CORTICAL CONTROL OF UNIT ACTIVITY IN THE CUNEATE NUCLEUS OF THE RACCOON. Mark J. Rowinski,* S. David Stoney, Jr.* (SPON: W. J. Jackson). Medical College of Georgia, Dept. of Physiology, Augusta, Ga. 30902

The effects of stimulating sensorimotor cortex on the activity of 188 neurons in the contralateral cuneate nucleus have been examined in 14 raccoons. The animals were anesthetized with pentobarbital and paralyzed with Flaxedil. In most experiments six cortical stimulation sites were used: distal forelimb motor, proximal forelimb motor, hindlimb motor, digital sensory, proximal arm sensory, and the cortical site producing the largest "P-wave" over the cuneate nucleus (area 3a). Cuneate units were classified according to their response characteristics to natural stimulation, responsiveness to stimulation of the contralateral medial lemniscus, and receipt of afferent "surround" inhibition. Of the total sample 64% were unaffected, 28% were inhibited, 1% were excited or facilitated, and 7% received mixed effects from sensorimotor cortex. Cortically influenced units were evenly distributed throughout the rostral-caudal and medial-lateral extent of the nucleus, across all submodalities of afferent input, and between cuneolemniscal relay and unidentified units. Area 3a and forelimb motor sites affected a significantly greater proportion of units than forelimb sensory and hindlimb motor cortical points. Many units were influenced from as many as all six cortical points. However, in comparing the effectiveness of the various cortical regions, a high degree of topographic specificity in the corticofugal influence over the cuneate was revealed. Best points for influencing units with distal receptive fields tended to be localized at cortical sites representing distal forelimb, while best points for influencing proximal units tended to cluster at proximal cortical sites.

1375 CENTRAL CONVERGENCE OF THE "COOL" THERMOSENSORY PATHWAY DEMONSTRATED PSYCHOPHYSICALLY. Andrew J. Rózsa and Dan R. Kenshalo. Dept. Psychology, Florida State University, Tallahassee, Florida 32306

A signal detection rating paradigm was used to test spatial summation of cool stimuli in 3 human observers. The stimuli were presented to two bilaterally symmetrical 18.4 cm^2 sites on each forearm. These sites were first preadapted to several temperatures (AT's) within and outside the temperature range of complete adaptation, before testing began. When the forearms were stimulated simultaneously, the detection level (d') was nearly identical to the d' of one forearm being stimulated at twice that intensity. This complete summation of cool stimuli occurred at all AT levels (24°C, 28°C, 32°C, & 40°C) and at all stimulus intensities, regardless whether the stimulus was near threshold (as low as -0.05°C) or clearly supraliminal (as high as -1.20°C).

Since peripheral nerves code information only from one side of the body, neural integration of sensory information from sites across the body midline must occur centrally. It is reasonable to assume that the mechanism of spatial summation of unitary areas is not different from that of two bilateral areas. The data reported here suggests therefore, that even if there is summation of cooling at the periphery, this peripheral information is either superseded or integrated at some central level in the nervous system.

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1376 INHIBITION OF NOCICEPTIVE REFLEXES IN THE PRIMATE BY ELECTRICAL STIMULATION OR NARCOTIC MICROINJECTION AT MEDIAL MESENCEPHALIC AND DIENCEPHALIC SITES: BEHAVIORAL AND ELECTROPHYSIOLOGICAL ANALYSES. M.A. Ruda, R.L. Hayes*, D.D. Price, J.W. Hu, & R. Dubner, NIDR, NIH, Bethesda, MD. 20014.

The effect of electrical stimulation or microinjection of a narcotic at midline brain sites on heat-evoked withdrawal reflexes was studied in awake monkeys. Electromyographic activity from tail and face was evoked only by noxious heat stimuli (45-51°C). Bipolar stimulation (1-5 ma, biphasic pulses at 20 hz for 20-120 sec) of some sites in the region of the central gray (CG) produced inhibition of tail flicks (TF) and lip twitches (LT). Inhibition often outlasted stimulation for 0.5-10 min. Naloxone (0.4-2.0 mg/kg), a narcotic antagonist, did not reverse the inhibition produced by CG stimulation in most cases. The injection of 5-40 µg of etorphine (10 µg/µl), a narcotic analgesic, into dorsal and lateral hypothalamic areas and caudal brain stem near CG produced reliable inhibition of TF and LT. The inhibition occurred 5-10 min post-injection, lasted 15-60 min, and was reversed by naloxone. The effect of CG stimulation on activity of projection and non-projection neurons in trigeminal nucleus caudalis (CAUD) was examined in monkeys previously tested behaviorally. Preliminary results suggest a preferential inhibition of responses to noxious stimuli in different classes of nociceptive and non-nociceptive neurons. The data indicate that (1) long-lasting inhibition of nociceptive reflexes commonly used to infer analgesia is mediated by both non-narcotic and narcotic systems of supraspinal origin, and (2) in agreement with previous reports, nociceptive input can be preferentially modulated at spinal cord and trigeminal brain stem sites by descending neural systems.

1377 SPINAL PROJECTION TO THE LATERAL AND CAUDAL PORTION OF THE PONTINE NUCLEI IN THE CAT. Dieter G. Rüegg*, Earl Eldred and Mario Wiesendanger. Institut de Physiologie, Univ. Fribourg, CH-1700 Fribourg, Switzerland.

Various lesions of the spinal cord resulted in a restricted area of degeneration in the most dorsal and lateral segment of the ipsilateral pontine nuclei. The spinal projection was also found to be confined to the most caudal portion of the pons. Hemisections (6 cats), ventral quadrant sections (5 cats) and dorsal quadrant sections (4 cats) were performed at high cervical or thoracic levels. The ensuing degeneration was studied with the Wiitanen technique. Degeneration was heavy after cervical hemisection. Ventral or dorsal quadrant lesions resulted in about equal amount of degeneration. A similar pattern of degeneration was observed after lesions at thoracic level. The area of degeneration was within the projection area obtained from large lesions of the ipsilateral pericruciate or SII cortical areas (5 control lesions). It is concluded that a small, well demarcated segment of the pontine nuclei receive spinal afferents from ipsilateral cervical and lumbosacral segments via fibres running in both, the ventral and the dorsal quadrant. This segment of the pontine nuclei thus represents another precerebellar relay for converging inputs from the cerebral cortex and the spinal cord.

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1378 DIFFERENCES IN AFFERENT FIBER POPULATION IN THE DORSAL FUNICULUS OF DOGS AT LUMBO-SACRAL AND CERVICAL SEGMENTAL LEVELS. Richard J. Schneider, Robert B. Bellegarrigue* and Thomas B. Ducker. Div. Neurosurg., Dept. Surg., Sch. Med., U. Md., Balto., Md. 21201.

Microelectrodes were used to record electrophysiological activity from the dorsal funiculus of mongrel dogs. From preliminary results in which more than 200 single fibers and multi-fiber records were sampled, three differences are apparent. Our sample at lumbo-sacral levels consists of 57% slowly adapting fibers while at cervical levels these fibers make up only 17% of the population. A second difference between these segmental levels is that while a lumbo-sacral plexus levels 65% of the fibers are related to physiological activity by muscles and joints, in the fasciculus gracilis at cervical plexus levels only 26% of the fibers can be so related. Thirdly, in the fasciculus gracilis, cervical segmental levels have a higher concentration of afferents from distal limbs (fore- and hindpaws) than do lumbo-sacral levels. Similar differences have been observed by us in the dorsal funiculi of experimental monkeys and by comparing the distribution of cervical afferents in the spinothalamic tract of humans undergoing percutaneous cordotomies with the results of open cordotomies and myelotomies in more caudal regions. We interpret these changes to imply that a re-arrangement of fibers occurs as one ascends the fasciculus gracilis from caudal to rostral levels, and that such rearrangements are a general rule for ascending afferent information in the spinal cord.

1379 NEURAL LOCUS OF SUMMATION IN THE WARMTH SENSE. Joseph C. Stevens. John B. Pierce Fndn. Lab., 290 Congress Ave., New Haven, CT 06519.

Spatial and temporal summation are two predominant properties of the warmth sense. The sense can integrate the neural effects of stimulation over hundreds of square centimeters and up to several seconds. These properties have been studied by at least three alternative psychophysical methods: threshold measurements, reaction time measurements, and measurement of sensation level as assessed by the method of magnitude estimation.

Is the neural locus of both kinds of summation peripheral, or central, or both? A partial answer to this question is that both kinds of summation can take place generously in the central nervous system. In one type of experiment it is shown that virtually the same degree of spatial summation takes place when stimulation is divided in two fields, one to either side of the midline, as when the stimulation is confined to a single side. The other type of experiment shows temporal summation (lowered thresholds; increase in subjective magnitude) when the first half of the stimulus is delivered to one side, the second half to the other side. The effect persists even when the two halves are separated temporally by as long as 0.75 sec, and its magnitude is approximately that obtained in control experiments on a single side.

1380 COMPARISON OF SENSORY DETECTION THRESHOLDS IN THE CAT WITH STIMULATION OF PRIMARY AFFERENT AND SUBCORTICAL SYSTEMS. John E. Swett and Charles M. Bourassa*. Dept. Anat. UC, Irvine, CA. 92664 and Dept. Psychol. Univ. Alberta, Edmonton, Canada.

We demonstrated in earlier studies that stimulation of skin primary afferents produced detection with intensities at, or slightly below, threshold for a recordable response in nerve or cerebral cortex. (J. Neurophysiol. 30, 515, 1967) Sensory detection thresholds were also normal with primary afferent inputs restricted to the dorsal column-medial lemniscus(DC-ML) system. (Brain Res. 70, 350, 1974) In the present study we compared the ability of normal animals to detect activation of the DC-ML system at its point of entry into the ventrobasal (VB) complex with primary afferent fiber activation in the same animals. Cats were trained to lever-press for food rewards in response to sensory cues produced from the two stimulus sites. Of the various subcortical sites tested VB was the most effective for eliciting behavior, but it was significantly less potent in this regard than stimulation of cutaneous primary afferents. Detection thresholds with VB stimulation declined with increased frequencies of stimulation but did not fall below 1.3-1.4 times the evoked response threshold in SI cortex. Stimulus rate had negligible effects on detection thresholds of the primary afferent fibers, thresholds being normally between 0.92 to 1.00T. Our results suggest that subcortical sites differ markedly in their relative abilities to elicit behavioral detection. VB may participate in the detection process, but other structures may play a more crucial role. (Supported by: NB02289, NS07493, NS07949, and MRC MA4112)

1381 DERMATOMAL MAPS OF THE FORELIMB OF MONKEYS. J.K. Terzis* and R.W. Dykes. Depts. Surgery and Physiology and Biophysics, Dalhousie Univ., Halifax, N.S., Canada B3H 4H7.

Several classical studies of the cutaneous distribution of the spinal roots have been done in the past century (Sherrington, Foerster, Head, and Keegan and Garret). Each has used behavioral or psychophysical methods of assessing the boundaries of the nerve distribution assuming that there was no central effect modifying the cutaneous input. Kirk and Denny-Brown (J. Comp. Neurol., 139:307, 1973) have shown this assumption to be in error; spinal mechanisms restrict the dermatomal map to about half of its true area. The peripheral distribution of the spinal roots serving the brachial plexus was investigated by the authors using single fibre dissection methods. The dermatomal maps obtained in this study were: (1) much larger than previously shown, and (2) subject to wide individual variations. These results have serious implications for the interpretation of peripheral nerve injuries and current methods of peripheral nerve repair. (Supported by the Medical Research Council of Canada)

1382 ROLE OF THE BRAIN STEM RETICULAR FORMATION IN THE MODULATION OF AROUSABILITY IN HIBERNATING GROUND SQUIRRELS. Delphi M. Toth, Dept. Anat., Univ. of New Mexico Sch. of Med., Albuquerque, N.M. 87131.

It is not known why an animal in deep hibernation, with a steady heart rate and body temperature, varies greatly in its arousability, as measured by its responsiveness to peripheral stimulation. The hibernator can be tested repeatedly for sensitivity to peripheral stimuli without necessarily causing complete arousal from hibernation. In the present study, the burst of muscle action potentials which may follow a stimulus was used as the index of the animal's responsiveness to stimulation. The brain stem reticular formation appears to be an area whose functional integrity is necessary for the hibernating ground squirrel to exhibit the erratic pattern of response characteristic of the intact hibernator. Responses to tactile stimuli were compared in unoperated hibernating Citellus lateralis and in hibernating ground squirrels with either cryoprobes or multiple recording microwires implanted chronically in the brain stem. When the neural activity of the reticular formation is cryogenically blocked, threshold to stimulation varies directly with body temperature, just as in the ground squirrel whose spinal cord has been transected at C_1 during hibernation. Previous studies have shown that hibernators with transection or ablation above the superior colliculi respond to stimuli as intact hibernators. Reticular formation activity, measured with large multiple unit electrodes and single unit microwires, increases when there is a dramatic shift in the level of arousal, as indicated by responsiveness to stimuli. Thus, the brain stem reticular formation appears to modulate arousability during hibernation in the ground squirrel.

1383 CORTICO-DORSAL COLUMN NUCLEI PROJECTIONS IN CATS AND MONKEYS. J.A. Weisberg and A. Rustioni. Depts. Anat. and Physiol. and Neurobiology Prog., Univ. North Carolina, Chapel Hill, N.C. 27514.

Cortical cells projecting to the DCN have been identified in cats using the retrograde transport of HRP (Weisberg and Rustioni,'76). In both species, cortico-DCN cells are layer V pyramids (no "giant" cells) in the sensorimotor cortex and in SII; they are numerous in areas 4 and 3a, and more sparse in 3b, 1 and 2 (Hassler and Muhs-Clement, '64; Powell and Mountcastle,'59). In monkeys, labelled cells are also concentrated in the supplementary motor area (Woolsey,'50). The question of whether cortical projections from different cytoarchitectonic areas terminate preferentially within the gracile-cuneate complex is being approached in adult cats in which restricted lesions are placed in the cortex and the degeneration is studied with the Fink-Heimer technique. Preliminary results show that fibers from the upper bank of the cruciate sulcus (mainly area 4) project mainly to the ventral regions and throughout the "transitional" area of the gracile n. Few, if any, fibers terminate in n. Z (relay for hindlimb Ia afferents). Fibers from area 4 in the lateral sigmoid gyrus terminate mainly in the ventral region of the cuneate n. (relay for forelimb Ia afferents). Less dense projections to both gracile and cuneate nuclei arise from caudal parts of the posterior sigmoid gyrus and from the anterior ectosylvian gyrus (SII). In agreement with previous observations (Kuypers and Tuerk,'64), cortico-DCN fibers terminate in nuclear areas characterized by multipolar cells and spare the "clusters" region. No appreciable sprouting into the "clusters" has been observed in either the gracile or cuneate nuclei of adult cats in which cortical lesions have been made one year after dorsal rhizotomy at lumbar (L_1-S_1) or brachial (C_3-T_1) levels.

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1384 UNIT ACTIVITY OF SUBSTANTIA GELATINOSA NEURONS: RELATION TO THE DORSAL ROOT POTENTIAL. G.L. Wilcox, M.W. Luttges and D.J. Mayer. Dept. of Physiology, Med. Col. of Va., Richmond, VA 23298, and Dept. of Aerospace Engineering Sciences, Univ. of Colo., Boulder, CO 80302.

The substantia gelatinosa (SG) has been implicated in the modulation of sensory impulses entering the dorsal horn of the spinal cord. Early indirect evidence suggested that SG activity evoked by dorsal root stimulation was involved in the generation of primary afferent depolarization (PAD) and the concomitant dorsal root potential (DRP). This study was designed to isolate multiple unit activity of SG in response to peripheral stimuli. Spatiotemporal correlation, an index of spatial heterogeneity of poststimulus time histograms (PSTH), was used to isolate responses of small SG neurons from those of nearby large neurons.

The lumbar spinal cord of mice was exposed by L3-L4 laminectomy under barbiturate anesthesia. Dual platinum microelectrodes (10 μM tip, 50-100 μM separation) were lowered through the dorsal horn in 25 μM steps. Amplitude discriminated PSTHs and averaged DRPs were accumulated in response to threshold electrical stimulation of the ipsilateral sciatic nerve. Correlation coefficients were computed across PSTHs from different electrodes within the same amplitude level. When the interelectrode correlation for the 50-100 μV range was plotted vs. depth, the minimum was repeatedly and exclusively found in the SG. PSTHs contributing to this minimum had a time course (20 ms latency) similar to and temporally predictive of the DRP (20 ms lead). Similar results were obtained from similarly prepared rats; electrical stimuli to both the ipsilateral and contralateral footpad evoked low amplitude SG activity; SG PSTHs correlated with the DRP. These data constitute the first direct evidence of SG spiking activity and its relation to the DRP. (Supported in part by USPHS Grants DE00116 and DA00576.)

1385 INHIBITION OF PRIMATE SPINOTHALAMIC NEURONS BY STIMULATION IN NUCLEUS RAPHE MAGNUS. W.D. Willis, J.E. Beall*, R.F. Martin* and A.E. Applebaum. Marine Biomedical Inst., Depts. Anat. & Physiol.-Biophys., Univ. Texas Medical Branch, Galveston, Texas 77550.

Electrical stimulation in midbrain or diencephalon reduces the behavioral responses to noxious but not tactile stimulation. This effect may be mediated by monoamine containing neurons. Stimulation in the raphe nuclei inhibits the activity of spinal neurons evoked by noxious inputs. It is therefore of interest to know if neurons belonging to the spinothalamic tract are similarly affected. Experiments were done on cynomolgus monkeys anesthetized with chlorolose and small amounts of pentobarbital. Spinothalamic tract neurons were identified by antidromic activation from the contralateral diencephalon. Low and high threshold neurons were distinguished by their responses to mechanical stimulation of hindlimb skin. Stimulation of the sural nerve evoked a split burst discharge due to the Aβ or Aδ components of the peripheral nerve volley. The nucleus raphe magnus was stimulated by an electrode inserted into the brainstem through the exposed floor of the fourth ventricle. Stimulus sites were confirmed histologically. Low and high threshold spinothalamic tract neurons were inhibited by brief trains (20 ms at 333-500 Hz) of stimuli applied in the nucleus raphe magnus. Threshold effects could be produced by stimulus currents less than 50 μA (0.1 ms pulses). Inhibition was maximum about 30 ms after the start of the stimulus train and lasted 150 ms. Aδ responses were depressed more than Aβ responses. Inhibition of spinothalamic tract neurons may contribute to the analgesic action of raphe stimulation.

(Supported by USPHS grant NS 09743 and Training Grant GM 00459.)

1386 NOXIOUS STIMULATION OF THE TOOTH PULP IN AWAKE CATS: A BEHAVIORAL STUDY. Stephen Wilson*, John R. Meyer, and Kenneth H. Reid. Dept. Physiology and Biophysics, UL, Louisville, Ky. 40201.

The jaw-opening-reflex and a shuttle response to tooth pulp stimulation in awake cats were studied. In this chronic study, bipolar stimulating electrodes were implanted in the upper canine teeth of cats. In addition, bipolar recording electrodes were implanted in the digastric muscles in order to monitor, electromyographically, the jaw-opening-reflex. Square wave pulses were applied to the teeth at a frequency of one pulse per second and the pulse duration was varied over a range of .1 to 2 msec. Stimulus strength (in volts) was incremented until both the jaw-opening-reflex and a shuttle response were obtained. The inter trial interval was approximately 30 minutes and daily sessions were run on each cat for several days. The range for elicitation of the jaw-opening-reflex was .18± .04 to 4.5± .52 volts. The range necessary to produce the shuttle response was 6.8± 4.43 to 31.6± 16.28 volts. This preliminary data indicated that the jaw-opening-reflex was less variable in comparison to the shuttle response which supports previous studies in our lab utilizing an excape paradigm.

1387 CODING PATTERNS OF LINGUAL THERMORECEPTORS IN THE CAT. Robert D. Wurster and F.-K. Pierau. Dept. of Physiology, Loyola University Medical Center, Maywood, IL. 60153; Max-Planck-Institut für physiologische und klinische Forschung. W. G. Kerckhoff Institut, Bad Neuheim, Fed. Republic Germany.

In cats anesthetized with α-chloralose, cold-sensitive thermoreceptor units were recorded from the lingual nerve. The tip of the tongue was placed on a servo-controlled water-perfused thermode. Temperatures were changed from 35 to 16 C in decrements of 1 to 2 C and each temperature was maintained for about 5 minutes. Firing patterns were analyzed during both the static and transient temperatures. A maximal mean static firing rate of about 8 impulses/sec occurred at 29 to 30 C. Occasional bursts of 2 impulses with an interburst interval between 20 to 40 msec usually began to occur in the temperature range of 26 to 30 C. At lower static temperatures the tendency to burst in pairs and the interval between bursts increased. Below 22 C the units occasionally demonstrated bursts of 3 impulses and the interburst interval exceeded 300 msec. The mean firing rate increased during the temperature transients. Single unit firing was rhythmic during temperature transients above 30 C, however the units demonstrated a tendency to fire in bursts during temperature transients below 30 C. Temperature transients below 20 C were characterized by bursts with as many as 5 impulses. Similar to the conclusions of Dykes (Br. RES. 98: 485-500, 1975), it is proposed that important information about cutaneous temperature could be transmitted to the CNS in the form of bursts at lower temperatures and during thermal transients. (Research supported by the Max-Planck Gesellschaft.)

1388 THE PROJECTION OF GROUP I AFFERENTS TO THE MOTOR CORTEX VIA AREA 3a.
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It is known that there exist separate projection areas for Group I muscle afferents in cortical area 3a and in the motor cortex.

To examine whether there are interconnections between these two foci in the cat, intracortical microstimulation (ICMS) was delivered to one of the foci and the evoked potential was recorded from surrounding areas including the other focus. It was found that there is a topographically specific interconnection of area 3a and the motor cortex. We then sought to determine if this specificity was related to Group I input to the motor cortex. Extracellular and intracellular recordings were made from neurons of area 3a while microstimulating within the deep radial (DR) nerve Group I focus of the motor cortex. The DR nerve and the pyramidal tract were also stimulated.

Most area 3a neurons antidromically activated by ICMS of the motor cortex received excitatory postsynaptic potentials from the DR nerve. Some of these neurons were also antidromically activated from the pyramidal tract. Neurons of the DR focus of area 3a were rarely antidromically activated from outside of the DR focus of the motor cortex.

We conclude that Group I input to area 3a is relayed to the motor cortex by these cortico-cortical fibers. (Supported by NIH grant # NS-10705).

Spinal Cord

1389 A RAT MODEL FOR THE INVESTIGATION OF SPINAL CORD INJURY

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A rat model for investigating spinal cord injury using the somatosensory evoked response (SER), and measurements of local spinal cord blood flow (SCBF) by hydrogen clearance technique is described. Rats are anesthetized with sodium pentobarbital IP and ketamine hydrochloride IM. Following tracheostomy and curarization, the animals are maintained on a respirator. Rats are placed in a stereotaxic apparatus, and the vertebral column immobilized. Bipolar recording electrodes are screwed into burr holes placed in the skull overlying the sensory cortex, and computer averaged SERs to electrical stimulation of an isolated peripheral nerve are recorded. After T10-12 laminectomy, a blood flow microelectrode is inserted into the spinal cord gray matter, and SCBF measurements taken. Systolic blood pressure, pulse rate, body temperature, and background EEG are monitored periodically. Reversible 50 gram-centimeter force (GCF) impact injury is applied to the exposed spinal cord segment in which SCBF is monitored. Immediately following injury, the SER disappears, but returns with wave component latencies increased, at variable times post-trauma (Table 1:A,B). SCBF decreases temporarily in response to trauma (Table 2). Experiments are continuing to further establish the relationship of the changes observed in the SER and SCBF to the pathologic changes resulting from trauma. To the best of our knowledge, this is the first description of a rat spinal injury model monitoring the above physiologic parameters. Our results are consistent with changes in SCBF following trauma observed in other animal models, and support the recent clinical use of the SER as a prognostic tool in the evaluation of spinal cord injuries in humans. We feel that this is an economical and reliable model for studying the pathophysiology and potential therapies of spinal cord injury.

TABLE I SER

		P1	N1	P2	N2	P3	N3
A.	X	9.31	20.77	38.76	64.23	87.54	122.04
	S.D. ±	1.43	2.50	4.76	9.71	12.22	11.59

mean latencies in msec for control SERs. n=60

		P1	N1	P2	N2	P3	N3
B.	X	26.19	54.81	88.96	130.10	167.91	200
	S.D. ±	6.93	2.10	10.21	6.03	16.86	

mean latencies for SERs returning after 50 GCF injury. n=8

TABLE II SCBF

	Control	Immediately	90 minutes	3 hours
X	49.42	36.58	33.91	48.31
S.D. ±	9.32	2.72	5.14	7.07
n	17	5	5	5

mean values of SCBF in ml/100 grams/minute observed during the control period, immediately following 50 GCF injury, 90 minutes and 3 hours post-injury.

1390 CLEARANCE OF LOCALLY ACCUMULATED EXTRACELLULAR POTASSIUM IN THE SPINAL CORD OF CATS. G. Cordingley and G. Somjen. Dept. of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

Subsequent to neural activity, significant amounts of excess potassium ions can accumulate in the extracellular space of the central nervous system. Active or passive processes, or both, may contribute to the clearance of locally accumulated extracellular potassium ($[K^+]_o$) during the several seconds following neural activity. The kinetics of excess potassium redistribution were studied with potassium-sensitive microelectrodes placed in the dorsal gray matter of the lumbar spinal cord of anemically decapitate cats. The initial resting level of $[K^+]_o$ was 3.23 ± .13 mM (mean ± s.e.m.) in cats which proved useful for experimentation. Electrical stimulation of afferent fibers in leg nerves produced elevations of $[K^+]_o$, where magnitudes of $[K^+]_o$ responses were positively related to frequency, duration, or voltage of the trains of electrical stimuli. $[K^+]_o$ usually returned to baseline within 5-10 seconds. The time required for clearance to half of the maximally elevated level (half-decay time) ranged from 0.55 to 1.9 seconds, and was positively related to both intensity and duration of repetitive stimulation. In a matrix of data in which both stimulus frequency and train duration were varied, half-decay times were consistently correlated with duration, but not with the magnitude of the response. Keeping duration and intensity constant, higher frequencies of stimulation were followed by identical or faster $[K^+]_o$ clearance than lower frequencies, even though the former produced the larger responses. The data were compared with the predictions derived from a model of an instantaneous cylindrical volume source of elevated $[K^+]_o$, for which diffusion equations are available[1]. The data were consistent with either of two explanations: 1) that diffusion processes alone are responsible for excess K^+ clearance, or 2) that diffusive processes are assisted by a relatively non-inducible active component. To distinguish between these possibilities, an inhibitor of Na^+,K^+-ATPase, digitoxigenin, was injected intravenously. Doses of 100 µg/kg produced reversible increases of clearance half-times of 13-25% without affecting the magnitude of K^+ accumulation. With larger doses $[K^+]_o$ redistribution was even more delayed and the effect was not reversible in 2 hours. These findings support the second explanation, that active pumping contributes to removal of excess K^+, but that the active contribution does not increase with increasing load, at least before half the load has been dissipated. (Supported by PHS grants NS 11933 and 5T01-GM-01678).

[1]J. Crank, The Mathematics of Diffusion. Oxford University Press: London, 1956, pp. 27-29.

1391 PSP PROJECTION PROFILES: A MEANS OF SUBCLASSIFYING MOTONEURONS. H. Wes Davis*, J. A. Farina*, and W. D. Letbetter.
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Recent studies indicate that the set of alpha motoneurons belonging to an individual muscle may be subclassified according to the specific physiological and histochemical properties of the individual muscle units which they innervate. (See Burke & Tsairis, 1974: Ann. N. Y. Acad. Sci. 228, 145) If these differences are expressed functionally by the selection of one or another "type" of motoneuron to participate in the type of motor performance most ideally suited to its own muscle unit's properties, then one might expect to find postsynaptic potentials (PSPs) from segmental reflex connections to be selectively distributed as well. Evidence supporting this concept has been published (Burke, et al., 1970: J. Physiol. 207, 709); however, techniques used in those studies require intact peripheral nerves and sensitive myographic recordings to characterize motor unit type. It would be useful if there were some simple test to differentiate motor unit types. The present study, then, was the first step in an attempt to establish such a test by investigating various PSP projections to large numbers of motoneurons belonging to the same motor nucleus in the same animal.

A computer-controlled data acquisition system provided a means for efficiently surveying the effects of repeatable graded afferent input to large numbers of motoneurons by using a graded stimulus strength series delivered to a large number of cut peripheral nerves in the hindlimb of anesthetized cats. PSPs were recorded intracellularly with glass microelectrodes from medial gastrocnemius motoneurons, and PSP projection profiles were obtained for each cell. Patterns of excitation, inhibition, or combinations of both varied according to which nerve was stimulated and at what strength it was stimulated. In addition, it was clear that the same group of electrically activated afferents from a given peripheral nerve projected different PSP patterns to individual members of the medial gastrocnemius motor nucleus. Furthermore, profiles obtained from different nerves (e.g., sural, plantar, and saphenous) suggested similar subdivisions among the same group of motor cells. The fact that sequentially recorded and adjacently positioned motoneurons could show entirely different PSP profiles refutes the idea that our results are artifacts of time of recording or of spatial distribution of neurons. It remains to be shown that our motoneuronal subdivisions have any relation to motor unit types thus far described by others; but if they do, the simple means of characterizing motoneurons by their PSP profile should provide a more specific framework within which to study motoneuronal and motor unit organization.

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1392 THE EFFECTS OF CHEMICAL AGENTS ON NEURAL REGENERATION FOLLOWING SPINAL CORD TRANSECTION IN RATS: A QUANTITATIVE STUDY. John B. Gelderd, Murray A. Matthews and Michele F. St. Onge*. Dept. of Anat., Sch. Med., L.S.U., New Orleans, La. 70119.

Male, Long-Evans hooded rats underwent spinal cord transection at the T-5 vertebral level. Following surgery, operated animals were separated into five experimental groups. Piromen was administered to the first group to reduce the amount of scar tissue at the injury site. An experimental tissue glue (isobutyl-2-cyanoacrilate) was topically administered at the lesion site to the second group to insure apposition of the severed tips of spinal cord and to achieve immediate hemostasis. The third group received ACTH to reduce inflammation at the injury site. The fourth group received Cytoxan which acts to suppress autoimmune responses. The fifth group served as non-treated controls. Operated animals were sacrificed at varying intervals from 7 to 180 days following surgery. Spinal cord at the site of lesion was sectioned horizontally and alternate slides were stained by the Bodian silver technique, then counterstained with cresyl violet and eosin. The site of lesion was assessed with respect to the density of the scar and the number of regenerating axons crossing the lesion site.

Although regenerating nerve fibers were observed within the dense scar in the non-treated controls, Piromen-treated animals showed a more diffuse scar and a three fold increase in the number of nerve fibers traversing the lesion when compared to controls. Cytoxan-treated animals revealed a two fold increase in the number of regenerating axons over control animals. Those animals treated with the tissue glue and ACTH showed no significant increase in regenerating nerve fibers over control animals. Invasion of the scar by peripheral nerves was seen in all experimental groups. These peripheral nerve contributions were predominantly seen within the lateral aspect of the scar. Cisterns were seen at the lesion site in both rostral and caudal stumps of spinal cord. These cisterns increased in size with time until they occupied almost the entire spinal cord volume immediately rostral and caudal to the lesion, forcing regenerating nerve fibers to the periphery of the spinal cord.
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1393 INVOLUNTARY MOVEMENTS OF THE UPPER LIMBS IN SPINAL MAN. S. Horenstein, M. Afroz*, P. Wichienkuer*, and R. Karis. Saint Louis University, Saint Louis, Missouri 63104.

Following transection of the spinal cord involuntary movements and postures of the upper limbs may emerge. In a series of patients with cervical spinal cord injuries resulting in severe degrees of transverse myelopathy, movements of portions of the upper limbs, usually finger flexion or finger and elbow extension, were observed in response to local and remote stimuli. Although these usually were seen in totally paralyzed muscles, they sometimes involved motor segments partially under voluntary control. They fell into four general groups: those provoked by mental effort such as arithmetic, others which followed neck movement usually sharp flexion, some specifically linked to involuntary spasms or movements of the lower limbs, and a variety induced in one upper or both lower limbs upon stimulation or movement of the other upper extremity. Those most frequently observed took place during strong flexor spasms of the lower limbs which appeared to spread to the arms serially involving segments from below upwards producing hip flexion, lateral trunk bending and finally one or more patterned movements of the upper limbs. When they spread to paralyzed segments they tended to persist for several seconds or even a minute or two after cessation of the lower limb spasm. Alternatively when they occurred in segments which were available to voluntary contraction, they pre-empted the muscle and prevented purposive movement. In contrast to the fixed limb postures attributed to "spinal dystonia," the upper limb muscles usually retained reflex function. The possibility of a transmitted Ia fiber component was assessed by stimulation of the popliteal nerve at threshold just below that required to evoke M waves. Even after repeated and prolonged stimulation no potentials were generated in the upper limb muscles and no organized responses occurred at lower limb levels. Touching the skin of the thigh over narrow or broad areas produced no visible or electromyographic alteration in the upper limbs, but rubbing the same area briskly as well as pricking it repetitively with a single pin or simultaneously with multiple pins evoked a few isolated discharges in those distant upper limb muscles likely to participate in involuntary movements even though gross flexor spasms did not occur. Cold had no effect. Squeezing the Achilles tendon readily produced EMG responses in upper limb muscles at variable latencies. Stretch of lower limb muscles and holding the deepest possible breath also set off upper limb activity. Kinesiological electromyography confirmed segment to segment ascent of such contractions. The latencies were highly variable and seemed shorter with more intense stimulation when units were also recruited rapidly. The use of one upper limb occasionally caused local contraction in muscles of the opposite arm. This was also noted on maximal stimulation of the ulnar and median nerves. Repeated examination during sleep in one patient failed to evoke any upper limb responses. The movements projected to the upper limbs from below and those generated in them appear most likely to depend upon the C fiber system as the adequate stimuli involve pain applied to broad areas of the body causing variably intense responses which occur sequentially and at differing latencies. The recruitment of upper limb motor units may be fractional. The basis for movements provoked by neck flexion is not clear but may relate to involvement of C fibers activated by stretching muscles and ligaments. The descending influences of mental effort and sleep appear to be facilitation of spinal motor neurons during the former state and either inhibition or defacilitation during the latter. Study of released upper limb responses in spinal man may expand the clinical assessment of the patient by indicating the degree of release of propriospinal reflexes and the extent to which descending influences remain capable of modifying local anterior horn cell function.

1394 DISTRIBUTION OF LISSAUER'S TRACT AND DORSAL ROOT FIBERS IN THE PRIMATE SPINAL CORD. Carole LaMotte. Department of Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Several series of experiments were conducted on the C7 segment of the Rhesus monkey spinal cord. The distribution of normal dorsal root afferents was mapped by labelling the C7 dorsal root ganglion with tritiated amino acids, and then compared with the degeneration of C7 dorsal root fibers following an intradural dorsal rhizotomy. To focus on the distribution of the small myelinated and nonmyelinated afferents, which include those serving pain and temperature sensibility, the degeneration following a Lissauer tractotomy was compared with the degeneration following selected lesions involving the large afferents and following dorsal rhizotomy. To facilitate identification of groups of fibers and terminals which might degenerate at different rates, the survival times of the lesions and rhizotomies were varied. Results showed:

1. Both large and small diameter dorsal root afferents exhibit the same rostro-caudal topography within the dorsal horn. The C7 root axons and terminals distribute throughout the mid-C7 dorsal horn grey. In sections proceeding rostrally through C6, the majority of the C7 root fibers ending in laminae I-IV shift to a lateral position. Proceeding caudally through C8, the C7 root fibers shift medially.
2. Very few small diameter C7 afferents entering via Lissauer's tract extend above C6 or below C8. Large diameter C7 afferents, arising as dorsal column collaterals, can extend at least to C4 above and T3 below, although diminishing greatly in number beyond C6 and C8.
3. For survival times of 1-10 days, autoradiography demonstrated label in all dorsal horn laminae, the heaviest occurring in the substantia gelatinosa. After 1 day, label was absent over dorsal column and Lissauer tract axons, suggesting that the dorsal horn label is mainly associated with fine axonal branches or possibly terminals. After 6-10 days many axons were labelled and could be traced into the dorsal and ventral horn.
4. Degeneration patterns from the rhizotomies and lesions, as demonstrated with Fink-Heimer and Nauta methods, depended on the survival time. No degeneration products were present before 3 days.
5. The large afferents degenerate within the dorsal horn after 3-4 days, contributing to the substantia gelatinosa, but mainly ending in laminae IV-VI. By 12 days, they can also be traced into the intermediate and ventral grey.
6. The tract of Lissauer consists of 2 populations, each containing small root afferents. One population degenerates at 3-5 days and distributes mainly to laminae II and III (substantia gelatinosa); the other degenerates around 12 days and distributes mainly to lamina I and the outer zone of II. It is suggested that the early degeneration may involve non-myelinated C afferents and the later degeneration may involve the small myelinated A delta afferents. (Supported by NIH RO3 MH27150-01).

1395 THE EFFECT OF CHRONIC DORSAL RHIZOTOMY UPON INTERNEURONAL ACTIVITY IN THE FELINE DORSAL HORN. Arthur Taub, L.M. Kitahata, M. Yamashita*.
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Six cats, male and female, 3.5-5.0 Kg in weight, underwent dorsal rhizotomy unilaterally at L6, L7 and S1, using microsurgical, aseptic technique, and were maintained for 18 months. None showed signs interpretable as dysethesia, though some incoordination of the ipsilateral lower extremity was evident. At the end of the maintenance period, spontaneous single unit activity in the dorsal and ventral spinal cord rostral to, within, and caudal to the level of the dorsal rhizotomy was studied. The technique was standardized to one previously used to establish control values for spontaneous activity in otherwise intact animals. It included bilateral mesencephalic reticular formation lesions, spinal cord transection at T12, core and spinal cord temperature servo-controlled at 37° C., femoral arterial blood pressure never below 80 torr, end-tidal pCO_2 between 3.5-5.5%, and a continuous infusion of gallamine triethiodide and 5% dextrose in saline. Vasopressors were never used. Spontaneous single unit activity was recorded upon magnetic tape. Receptive field configuration, modality responsiveness, and response to 75% N_2O/O_2 was determined. Interval histograms were determined off-line. For each unit at least 10 such histograms were averaged bin-by-bin and a mean histogram with a bin-by-bin standard error was derived and displayed. Major recording sites were localized through thermal lesions. Spinal cords were fixed in situ with formalin and stained with cresyl violet. The activity of 75 single units were studied and compared with that of 95 control single units and histogram averages.

There was almost complete absence of single unit activity in Rexed laminae 4 and 5 adjacent to the rhizotomy site. More ventral laminae showed persistent but slowed activity, altered in pattern. Some minor increase in spontaneous activity was found contralateral to the lesion. The region of "electrical silence" could be correlated pathologically with a region of intense gliosis, spinal cord atrophy, and possible transynaptic degeneration of dorsal horn neurons, particularly in laminae 4 and 5.

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1396 SPINAL MECHANISM OF MORPHINE ANALGESIA. H. Toyooka*, L. M. Kitahata M. Yamashita*, K. Hanaoka*, M. Ohtani*, A Taub. Dept. Anes., Yale Univ. Sch. Med., New Haven, Conn. 06510.

The spinothalamic tract is believed to be a pathway significant for transmission of information necessary for pain and temperature sensation in man. Some of the cells of origin of the spinothalamic tract can be recorded from microelectrodes. They may be located in the spinal cord by antidromically activating their thalamic axonal extensions. In this way they were located in Rexed laminae VII and VIII of feline lumbar spinal cord by Dilly, et al., Trevino, et al., and Levante and Albe-Fessard.

Recently it has been shown that morphine and its surrogates exert suppressive effects upon nociceptive neurons in the spinal cord as well as the brain stem structure. It is of significance, therefore, to study the effects of morphine-sulphate on the activity of single units in Rexed lamina VII which are considered to be the major cells of origin of the spinal thalamic tract and which respond principally to noxious stimuli.

METHODS. Under halothane anesthesia cats were prepared with midcollicular decerebration, lumbar laminectomy, and upper lumbar spinal cord transection. Anesthetics were then discontinued and the animals were artificially ventilated. All physiological parameters were maintained within normal limits. Cells in lamina VII were sampled with microelectrodes 500-1000 microns ventral to lamina VI.

Following the recording of spontaneous firing of the unit during the control period, 0.5 mg/kg of morphine-sulfate was administered intravenously and the effect was observed for 90-120 minutes until complete recovery was observed. In some animals naloxone 0.01 mg/kg was administered intravenously to see its antagonistic effect. A similar experiment was repeated utilizing 1.0 mg/kg of morphine-sulphate. At the end of the experiment an electrolytic lesion was made by passing dc current through the recording electrode and its lesions were verified histologically.

RESULTS. Morphine-sulphate showed a significant suppressive effect on the spontaneous firing frequency of single unit activity of cells in Rexed lamina VII, 60 and 70 per cent suppression with 0.5 and 1.0 mg/kg of morphine respectively. The degree and duration of suppression were significantly greater than that on the single unit activity of cells in laminae I and V, also associated with nociception. Naloxone 0.01 mg/kg i.v. reversed the morphine suppression completely.

DISCUSSION. The significance of the present study is the finding that morphine-sulphate in therapeutic concentrations exerts a significant suppressive effect on the activity of those neurons in Rexed lamina VII which are associated with nociception. The degree and duration of suppression by morphine sulphate on cells in Rexed lamina VII was greater than that exerted on cells in laminae I and V, also associated with nociception. The suppressive effect of morphine on the cells in lamina VII are not centrally mediated, as the spinal cord was transected at the upper lumbar level.

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1397 MICROSPHERE DETERMINATION OF LAMINECTOMY INDUCED CHANGES IN SPINAL CORD BLOOD FLOW. Douglas K. Anderson, Gregory R. Nicolosi*, Eugene D. Means, and Lawrence E. Hartley*. Research Service, Tampa VA Hosp. and Dept. of Physiol. and Med., Univ. South Florida Coll. Med., Tampa, Fl. 33612

We have previously reported in cats that immediately following a one segment laminectomy at L-2, spinal cord blood flow (SCBF) was depressed 25-40% below normal along the entire length of the spinal cord (SC) as measured by the reference sample method using isotope labelled microspheres (Nicolosi et al. Fed. Proc. 35: 448, 1976). In order to determine if this laminectomy-induced flow alteration was prolonged, cat SCBF was measured at a longer time interval following laminectomy. A control dose of microspheres was injected into the left atrium, a one-segment laminectomy performed at L-2, and 24 hours later, a differently labelled dose of microspheres was administered by the same route. A one-minute reference blood sample was withdrawn from the thoracic aorta during each microsphere injection. Following the second dose of microspheres the entire SC was removed, sectioned into 2 cm segments, weighed and the activity determined along with the blood samples. Control flows for the feline SC ranged from 15.0 ml/min/100 gm in the thoracic SC to 24.1 ml/min/100 gm in the cervical and lumbar SC enlargements. At 24 hours post-laminectomy there was a significant elevation in SCBF ranging between 15-35% above control along the entire length of the SC. Although SCBF was depressed immediately following laminectomy, by 24 hours, SCBF had risen to levels exceeding those for control. At the present time the duration of and the mechanism(s) for the post-laminectomy SCBF change remains unknown. Transverse paraffin embedded sections (20 μm) from various levels of the SC were stained with hematoxylin and eosin. Microspheres were noted in capillary sized vessels but these produced no observable histological abnormalities. (Supported in part by the Hillsborough County Heart Assoc., a chapter of the Florida Heart Association.)

1398 SEGMENTAL REFLEXES AND CSF HVA, MHPG AND 5HIAA FOLLOWING LESIONS OF THE SPINAL CORD IN MAN. Peter Ashby, Molly Verrier*, Jerry J. Warsh* and Kathleen S. Price*. Univ. Toronto, Toronto, Ontario, M5S 1A8, Canada.

Descending bulbospinal pathways, that employ specific neurotransmitter substances, are known to be capable of modulating segmental reflex activity in the experimental animal. To determine whether this might also occur in man correlations have been sought between the activity in spinal reflex pathways and the lumbar cerebrospinal fluid (CSF) concentrations of 5 hydroxyindolacetic acid (5HIAA), 3 methoxy-4-hydroxyphenylglycol (MHPG), and homovanillic acid (HVA) in 12 patients with complete or virtually complete spinal lesions.

The concentrations of 5HIAA and MHPG in lumbar CSF are reduced following complete or virtually complete spinal lesions in man. This may occur within 18 days of the lesion. MHPG concentrations appear to be inversely related to the level of the lesion.

The HVA concentration in lumbar CSF is reduced when there is obstruction of the CSF pathways.

No relationship could be demonstrated between the concentrations of 5HIAA or MHPG in lumbar CSF and the activity in the spinal monosynaptic pathway (estimated from the proportion of the motoneurone pool activated by the Achilles tendon reflex (ATR) or H reflex) or the activity of a spinal inhibitory mechanism (estimated by the degree of vibratory inhibition of the monosynaptic reflex).

Patients with a tonic vibration reflex (TVR) tended to have higher MHPG levels. There appeared to be an association between low CSF HVA and enhanced vibratory inhibition of the monosynaptic reflex in the 9 patients whose spinal lesions were complete.

1399 ELECTROLYTE DISTRIBUTION AND STABILITY OF THE ISOLATED FROG SPINAL CORD. C. P. Bianchi and S. D. Erulkar. Dept. Pharmacol., Univ. Pennsylvania, Philadelphia, Pa. 19174.

Electrolyte analyses of isolated frog spinal cords equilibrated with oxygenated Ringer's solution reveal that the spinal cord is in steady state conditions from 3 to 24 hours. The electrolyte content of spinal cords equilibrated for 3-6 hrs (N=12) was as follows: μmol/g, K^+, 70.3±3.3; Na^+, 44.3±1.7; Mg^{2+}, 4.74±0.27; Ca^{2+}, 2.64±0.4; and Zn^{2+}, 0.29±0.01; for 24 hours (N=5); μmol/g, K^+, 65.5±2.1; Na^+, 35.2±0.7; Mg^{2+}, 5.50±0.87; Ca^{2+}, 2.14±0.41. Sodium and chloride contents were found to be 46.2±1.2 μmol/g vs 46.6±1.4 μmol/g respectively. The sodium and chloride spaces were not significantly different from the sucrose space. Analyses of the kinetics of ^{22}Na washout reveal that 2-6 μmol/g of sodium may be in a slowly exchanging compartment. Kinetics of calcium washout show that 1/3 of the total calcium content is lost within 2 minutes; the remainder is washed out with a time constant of 333 minutes. These results show that the isolated frog spinal cord is in steady state conditions for up to 24 hours and that intracellular sodium and chloride are exceedingly low and completely exchangeable. The low internal chloride may account for the failure to record sustained hyperpolarizing potentials from frog spinal neurons (supported by NS00321 and NS12211).

1400 EFFECT OF SPINAL CORD INJURY ON CORD BLOOD FLOW IN MONKEY. W.G. Bingham, L. Sirinek*, K. Crutcher, C. Mohnacky*. Div. Neurosurgery and Dept. Anatomy, Ohio State Univ. Med. Sch., Columbus, Ohio 43210

400 gram cm blunt trauma to T6 segment of adult male monkey spinal cord resulted in severe paraplegia, hemorrhagic necrosis of central gray matter and altered blood flow throughout the entire spinal cord. Using Cl4-antipyrine indicator fractionation technique blood flow was measured in traumatized segment and in several intact segments and was expressed as a function of time 5 min to 12 hrs post trauma. Cardiac output, mean arterial pressure, blood gasses, and end expiratory CO2 were recorded. Flow in traumatized central gray matter was obliterated by trauma, white matter blood flow at T6 fell to 50% of normal values in 30 min then rose gradually over the next 12 hrs. Flow throughout the remainder of the cord fell to 58% in the cervical area, 60% at T2 and 59% in the lumbar area. Gray:white flow ratios in intact cord remained constant indicating flow in each compartment was affected equally. Mean arterial pressure did not fall significantly, however a significant decrease in cardiac output occurred. Autoregulation of the entire cord appears to be affected by localized trauma to a mid thoracic segment. Sequestration of the circulating blood volume may be a contributing factor.

1401 ANATOMY OF THE MEDIAL GASTROCNEMIUS AND SOLEUS MOTOR NUCLEI IN THE CAT
R.E.Burke, K.Kanda*, B.Walmsley*, P.Strick and C.C.Kim*. Lab. of Neural Control, NINCDS and Lab. of Neurophysiology, NIMH, NIH, Bethesda, MD.

Retrograde transport of horseradish peroxidase (HRP) was used to label motoneurons innervating either the medial gastrocnemius (MG) or soleus (SOL) muscles in 4 adult (1.5 - 2.0 kg) female cats. Using anesthesia and sterile conditions, we injected 50 mg of HRP (type VI, Sigma) in 1.0 ml saline throughout the left MG muscle and 25 mg HRP in 0.5 ml saline into the right SOL. In 2 cats, nerves to contiguous muscles were cut before MG injection. After 4 days, animals were re-anesthetized and perfused with glutaraldehyde-paraformaldehyde fixative. Serial frozen sections (sagittal plane; 50 um thick) of spinal segments L7-S1 were processed conventionally. The positions of cell bodies with nucleoli were plotted and their major and minor diameters measured. Reconstructions in 2 cats showed, respectively, 360 and 337 labeled MG neurons and 186 and 181 SOL cells. Histograms of average soma diameters were bimodal, with 25.2% to 29% of motoneurons in the gamma size range (15 - 38 um average diameter). Most gamma motoneurons were more heavily labeled, and had larger granules, than cells in the alpha size range (38.5 - 80 um). MG and SOL motor nuclei were flattened columns (MG length: 7.4 - 9 mm; SOL length: 6.6 - 8.2 mm) less than 1 mm wide at maximum. Cell densities were greatest in the middle 1/3. Alpha and gamma motoneurons were intermingled but the rostral 1/3 of the MG nucleus showed a disproportionately high percentage of large alpha cells ($>$55 um diameter). In the caudal region of nuclear overlap, $>$95% of all the neurons present were either MG or SOL motoneurons. Thus, retrograde transport of HRP appears to label most if not all alpha and gamma motoneurons under these conditions, permitting detailed study of the anatomy of individual motor nuclei.

1402 A PROJECTION FROM UPPER CERVICAL SPINAL CORD TO IPSILATERAL THALAMUS IN CAT AND MONKEY: A NEW SOMATOSENSORY RELAY FROM THE BODY?
Earl Carstens* and Daniel L. Trevino. Neurobiol. Program and Dept. Physiol., U. of N. Carolina Sch. Med., Chapel Hill, NC 27514.

In a study of the locations of the cells of origin of the spinothalamic tract using the method of retrograde axonal transport of horseradish peroxidase (HRP; LaVail & LaVail, Science:1416, 1972), the locations of retrogradely labeled neurons in the second cervical segment (C-2) were determined after large HRP injections (3 µl) into the caudal thalamus. In addition to expected neuronal labeling in the dorsal horn and lateral cervical nucleus contralateral to the injection site, many labeled neurons were seen in the lateral part of Rexed's laminae VII-VIII on the side ipsilateral to the injection site in 3 cats and one rhesus monkey. These small and medium-sized ipsilateral neurons were not organized as a distinct nucleus, but were scattered along the rostrocaudal axis of C-2. A small number of labeled neurons was seen in the same region on the contralateral side. Small HRP injections (0.3 µl) into various thalamic nuclei in cats (centralis lateralis, rostral ventrobasal-ventralis lateralis, magnocellular medial geniculate), and into the mesencephalic periaqueductal gray of one monkey, also resulted in the labeling of neurons in ipsilateral laminae VII-VIII. These neurons may therefore project widely throughout the thalamus.

In an ongoing, complementary study of the function of these previously undescribed ipsilateral spinothalamic neurons in C-2, the activity of single units in C-2 which are antidromically excited from the ipsilateral thalamus is being recorded with tungsten or glass microelectrodes in anesthetized paralyzed cats. The 12 units studied to date commonly had receptive fields on the back and tail, often including all four limbs. Adequate cutaneous stimuli ranged from tap or firm pressure to noxious pinch.

1403 THE ABSENCE OF UNMYELINATED FIBERS IN THE SPINAL ROOTS AND NERVES OF THE STINGRAY. R.E. Coggeshall, M.L. Applebaum*, R.B. Leonard and W.D. Willis. Marine Biomedical Inst., Depts. Anat. & Physiol. - Biophys., Univ. Texas Med. Br., Galveston, Tex. 77550.

The dorsal roots of all vertebrates investigated heretofore contain large numbers of unmyelinated fibers, and there is increasing evidence of a substantial ventral root unmyelinated component as well. Thus, it was a surprise to find that an elasmobranch fish possessed essentially no unmyelinated fibers in the dorsal roots, ventral roots, or peripheral nerves. The atlantic stingray (Dasyatis sabina) has more than eighty sets of spinal roots. After anesthesia in MS-222, the spinal roots and nerves were fixed either by immersion or perfusion. In three rays, every fifth peripheral nerve and every tenth pair of dorsal and ventral roots were examined in the electron microscope. The numbers of myelinated fibers were variable but averaged approximately 1500 in the dorsal root and sensory part of the peripheral nerve and 700-800 in the ventral root and motor part of the peripheral nerve. By contrast the unmyelinated fibers in these same nerves and roots numbered less than 0.6% of the myelinated fibers and many of the dorsal roots and peripheral nerves had no unmyelinated fibers. Histograms showed that the dorsal root and its spinal nerve continuation contained large and small myelinated fibers whereas the ventral root and its spinal nerve continuation contained predominantly large myelinated fibers. Compound action potentials obtained from these same roots and nerves were consistent in that the sensory volly had two peaks, the motor volley one peak and there was no evidence of a C fiber response. We conclude, therefore, that almost all sensory information reaching the spinal cord in stingrays is carried by myelinated fibers. This arrangement may provide insights into the role of unmyelinated fibers in higher animals (Supported by UPPHS grant NS 11255).

1404 THYMECTOMY, BOTH ALONE AND IN COMBINATION WITH PHARMACOLOGIC IMMUNOSUPPRESSION, TO ENHANCE SPINAL CORD REGENERATION IN THE RAT. Robert C. Dauser*, Earl R. Feringa, and H. Lee Vahlsing*. Dept. Neurol., Univ. of Mich. Med. Cent. and Neurol. Serv., VA Hosp., Ann Arbor, MI 48105.

Isogeneic female rats were used to study the effect of immunosuppression on spinal cord regeneration. Thymectomy was performed on 50 rats within 24 hours after birth. The mid-thoracic spinal cord was transected at 6 weeks of age. No further treatment was given to 25 animals while the other 25 received an intraperitoneal injection of 75 mg/kg of cyclophosphamide within one hour after transection. A control group of 25 animals without thymectomy had spinal cord transections and no drug treatment.

All animals were evaluated electrophysiologically by stimulation of the long descending motor tracts in the high cervical cord, with simultaneous recording of potentials off ventral roots below the transection site. Neither direct observation nor computer of average transients analysis of recorded responses showed any evidence for regeneration.

In half the animals from each group, radioactive proline was injected into the motor cortex 21 days prior to electrical testing and sacrifice. Evaluation of radioactivity in the lumbar segment of the cord also failed to show evidence of long motor tract regeneration.

In the other half of the animals from each group, a horseradish peroxidase (HRP) pellet was implanted in a fresh gap made in the spinal cord below the original transection site, at the time of electrophysiologic testing. Histologic sections of the motor cortex were made three days later to search for HRP which would have traveled there via retrograde transport up motor axons traversing the original transection site. No motor cortical cells showed obvious HRP in their cell bodies.

1405 HISTOLOGICAL EVIDENCE OF THE EFFECTIVENESS OF IMMUNOSUPPRESSION IN PROMOTING SPINAL CORD REGENERATION IN RATS. Sam W. Davis*, Earl R. Feringa, and H. Lee Vahlsing*. Dept. Neurology and Pathology, Ann Arbor VA Hospital and Univ. of Michigan, Ann Arbor, MI 48105.

Forty-one isogenic rats, 6 weeks of age, were used to evaluate the effectiveness of an immunosuppressant drug, Cytoxan, for enhancing spinal cord regeneration after complete cord transection at the mid-thoracic level. Sixteen animals were treated with 1 I.P. dose of 75 mg/kg Cytoxan at 1 hour post transection; 12 were treated similarly at 24 hours post transection; and 13 control animals received I.P. isotonic saline after transection.

Six months later all animals were tested for electrophysiological evidence of regeneration by stimulation of their corticospinal tract at a level 2-4 vertebral segments above the previous transection site while recording responses shown in their ipsilateral sciatic nerve. No significant differences were found between treatment and control groups.

After physiological testing each animal's cord was retransected at the stimulation site. Five-7 days later the animals were killed and their cords were evaluated using the Fink-Heimer/Nauta stain. Sections from each cord between the new and old transection sites served as positive controls. Sections from either the lower thoracic or upper lumbar cord caudal to the first transection site were examined for evidence of recent degeneration of axons. Such fiber degeneration 5-7 days after the new and higher cord transection is evidence that regeneration of descending fibers had occurred in the 6 month interval. No significant difference was found between Cytoxan at 1-hr. and control groups. However, the animals treated with Cytoxan at 24-hr. post transection showed increased regeneration over the control animals using the student's T-test ($p = 0.03$).

1406 LONG LASTING CHANGES IN THE MONOSYNAPTIC REFLEX PRODUCED BY DORSAL COLUMN HEMISECTION. E. E. Decima, M. Ariano* and F. R. Morales*. Anat. Dept., School of Medicine, UCLA, Los Angeles, CA. 90024.

The aim of this study was to analyze the changes present in the synapses, made by a given axon, some time after a branch of the same axon had been severed. The system used for this purpose was the monosynaptic reflex (MR) of the rat lumbar cord (i.e., the Ia fiber--α motoneuron synapses). The preparatory surgery consisted of severing a branch of the Ia fibers after they had entered into the cord. This was achieved by section of the dorsal column in one side (i.e., cutting the ascending branch of the DR fibers) at the level of L2-L3. At variable post-operative periods the MR of the L4 (or L5) segment was studied simultaneously in both sides of the cord (the unoperated side thus serving as a control). In the first few days after the operation the MR is very small in the operated side and fatigues very rapidly even to frequencies of stimulation as low as 0.5 Hz. After 3 weeks the MR is almost normal in size. A clear change in its curve of post-tetanic potentiation (PTP) appears at this time: the maximum potentiation is much larger and lasts longer in the operated side as compared with the contralateral control. This effect in the PTP of the MR has been observed to be present up to 70 days of the post-operative period.

Results similar to these were obtained by Eccles & McIntyre (J. Physiol., 1953, 121:492) after sectioning the DR's distal to the spinal ganglion in the cat lumbar cord. Possible interpretations of this phenomenon will be discussed.

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1407 A SIMPLE METHOD FOR THE REMOVAL OF MOUSE AND RAT SPINAL CORD. Byron N. de Sousa and Lloyd A. Horrocks. Department of Physiological Chemistry, The Ohio State University, Columbus, OH. 43210.

A simple, fast and inexpensive method for the removal of rat and mouse spinal cord is described. This method enable us to remove either rat or mouse spinal cord within one minute, following the animal decapitation, hence reducing the "normal" time of removal by 20 to 30 times. Animals are sacrificed by decapitation in a cold room at 4°C. The approach to the vertebral canal is accomplished through its caudal end, where a hypodermic needle mounted on a syringe filled with water is inserted. Pressure is exerted on the syringe plunger to force the spinal cord out of the vertebral canal through its cervical portion. Some rat spinal cord parameters such as weight and length during maturation have been studied. Rat spinal cord increased markedly in weight and length during development. The spinal cord weight was 34 mg at birth and its length was 25 mm, vs. 700mg and 110 mm at adult age. It is hoped that this method will stimulate further, more systematic research on the neurochemistry of the spinal cord.

1408 CORRELATION BETWEEN PRIMARY AFFERENT DEPOLARIZATION (PAD), LOCALIZATION OF ENDOGENOUS GABA, AND GLUTAMIC AND DECARBOXYLASE (GAD) IN THE FROG SPINAL CORD. S. Glusman, D. Mc Adoo, P. Rudomín and B. Haber. Dept. of Physiology, Centro de Investigación del Instituto Politécnico Nacional, México 14, D. F. and The Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Tx. 77550.

The findings of a close parallelism between the intraspinal distribution of GABA uptake and PAD in the frog spinal cord suggests that elements that take up GABA are involved in the generation of PAD. The posible involment of GABA in PAD would be further strengthened if the biosynthetic enzyme GAD and the levels of GABA per se might show a similar spatial correlation as does the uptake of GABA. In these studies frog spinal hemicords were incubated with L-U-C^{14} glutamic acid and then sectioned in a dorsal-ventral direction (200 μ sections). The percentage of labelled GABA formed in every slice was taken as an index of GAD activity. Levels of labelled GABA was significantly higher in the dorsal region. The levels of GABA, as well as those of glycine, glutamic and aspartic acids were determined in extracts of 100 μ hemicord sections by gas chromatography-mass spectrometry. Greatest amounts of GABA were found at 400-500 u from the dorsal surface coincident with maximal GAD activity, sites of GABA uptake, and current sink generated during PAD. On the other hand glycine, aspartate and glutamate were more concentrated in the ventral regions. The presence of axo-axonic synapses which are GAD positive by inmunohistochemical techniques in the dorsal areas of the spinal cords as shown by others taken together with the above findings strongly suggest a role for GABA in the generation of PAD.

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1409 THE ULTRASTRUCTURAL IDENTIFICATION OF PRIMARY AND SUPRASEGMENTAL AFFERENTS IN THE MARGINAL AND GELATINOUS LAYERS OF LUMBAR SPINAL CORD FOLLOWING CENTRAL AND PERIPHERAL LESIONS. G.E. Goode. Department of Anatomy, The Ohio State University, Columbus, Ohio, 43210.

The glomeruli which characterize the substantia gelatinosa (SG) of the opossum lumbar spinal cord, contain a central (C) primary afferent terminal surrounded by multiple pre- and postsynaptic elements. The C terminal is presynaptic to dendritic shafts and two types of dendritic spines. Type 1 dendritic spines are the major postsynaptic elements of C terminals and participate in dendrodendritic synapses with type 2 dendritic spines as well (type 2 spines contain ovoid synaptic vesicles). Some C terminals degenerate following dorsal rhizotomy (Goode, Anat. Rec., 184:412, 1976). These primary afferents are sensitive also to peripheral nerve lesions and darken two weeks subsequent to skin excision. Degenerating axons are found in the dorsolateral fasciculus subsequent to peripheral nerve lesion but do not appear in the dorsal columns. Although a few glomeruli are present in lamina I, the marginal layer contains many extra-glomerular terminals with granular synaptic vesicles. The axoplasm of large, granular terminals in the marginal and SG layers "darken" around clumped synaptic vesicles 48 hours after rostral pontine and cervical hemisection. Glial cytoplasm isolate some of these darkened terminals 9 days postoperatively. Although postsynaptic elements are rare, a few terminals contact small dendritic shafts in the marginal layer. These data suggest that (1) the primary afferents which are sensitive to peripheral nerve lesions are the small fiber component of dorsal roots and (2) terminals which contain granular vesicles in the marginal and gelatinous layers originate from rostral as well as caudal brainstem.
This investigation was supported by General Research Support Grant 5409, NIH.

1410 BURST FIRING PATTERNS WITHIN THE SPINAL CORD DORSAL HORN OF CHICKEN (G.domesticus). J.A. Holloway, L.E. Wright, C.O. Trouth, and G-M. Moolenaar. Dept. of Physiol.,Coll. Med. Howard Univ., Wash.,D.C.,20059

The firing pattern of spinal cord dorsal horn cells (DHC) of lightly nembutalized chickens has been examined, focusing upon both the spontaneous firing patterns and modification of these patterns by natural stimuli. Most of these units exhibited stereotyped doublet or triplet burst firing patterns. The interspike interval was 2 ± 1 msec in a given cell. The triplet pattern occurred most frequently, with an interburst interval of $60\text{-}70\pm2$ msec. The less frequent doublets almost always generated the following pattern: two pairs with interburst interval of $20\text{-}30\pm1$ msec followed by another doublet or pair of doublets with intervals of $50\text{-}55\pm1$ msec; few extended to 60 msec. When the DHC were driven by light touch, feather movement, or ethyl chloride spray on the ipsilateral body wall, three responses occurred. 1) firing rate increased and the proportion of spikes occurring within bursts ("burst index") increased. 2) firing rate increased but burst pattern was disrupted. 3) increase in frequency of doublets with a decrease in frequency of triplets. If a noxious stimulus (pinch) was applied to the comb during spontaneous activity or during evoked activity, units were totally inhibited. These findings suggest: a) that triplet burst firing patterns are more likely at low levels of activity and decrease with increased activity which may imply an automatic gain control in the repetitive firing mechanism of neurons. b) that this burst pattern represents a "unique" form of coding in the chicken CNS. (Supp'td. by NIGMS Trng. Grant 1 TO 2 GM-05010-01.

1411 THE CONCENTRATION AND LOCALIZATION OF CATECHOLAMINES IN THE SPINAL CORD OF THE SUBHUMAN PRIMATE. J.D. Irvin, E.T. Angelakos, J.L. Alderman and I. Mohler*. Hahnemann Med. Col., Philadelphia, PA. 19102 and Jefferson Med. Col., Philadelphia, PA. 19107.

Biochemical, radioenzymatic and histofluorescent techniques were employed to study the segmental and intrasegmental concentration and specific localization of catecholamines (CA) in the spinal cord of the African Green Monkey (Cercopithecus aethiops). Male animals were anesthetized with ketamine, sacrificed by transection of the thoracic aorta, and the spinal cord was excised. The leptomininges were dissected free of the cord parenchyma. The greatest concentration of norepinephrine (NE) was found in the upper cervical (C_1-C_4) and the lumbar segments of the cord. The concentration of NE in these regions was approximately twice that found in the remaining cervical (C_5-C_8), thoracic and sacral segments. Results from the more sensitive radioenzymatic method are in agreement with these findings. In addition, this later method demonstrated an intrasegmental variation in the catecholamine content in the lumbar segments, with the highest concentration in the midsegmental region. Histofluorescent studies demonstrated the presence of catecholamine containing varicosities at both the tissue parenchymal and vascular levels. The parenchymal fluorescence was most abundant in the thoracolumbar intermediolateral gray (ILG) and the cervical posterior gray regions. A commissural band of CA fluorescence extending between the ILG and lying dorsal to the spinal canal was seen in the low thoracic regions. The cervical posterior gray fluorescence was increased markedly when tissues were reacted under conditions used to demonstrate the presence of secondary catecholamines (epinephrine). White matter CA fluorescence is present in association with the tissue vasculature. These studies indicate an extensive and specific distribution of adrenergic nerve terminals in all regions of the spinal cord in subhuman primates. Supported by NIH HL 13008.

1412 DIFFERENTIAL RECRUITMENT OF MEDIAL GASTROCNEMIUS MOTOR UNITS PRODUCED BY CUTANEOUS (SURAL NERVE) INPUT IN DECEREBRATE CATS. K.Kanda*, B.Walmsley* and R.E.Burke. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

In the cat, the medial gastrocnemius (MG) muscle contains a mixture of fast and slow twitch motor units while the synergistic soleus (SOL) has only slow units. In most precollicular decerebrate cats, tetanic stimulation of the ipsilateral sural nerve (100 hz, 2-3 X electrical threshold), superimposed on a tonic vibration reflex (TVR) in MG and SOL produced by longitudinal vibration (160 hz, 60-150 um) of either or both muscles, generates markedly increased whole muscle tension and EMG activity in the MG and simultaneously inhibits tension and EMG activity in SOL. This observation plus previous evidence (Burke et al., J.Physiol. 207:709,1970) led us to examine whether, under similar conditions, motor units within the MG population show differential recruitment. The activity of individual MG motor units was monitored by EMG recording from the muscle (fine bipolar wires) or by recording small numbers of motor axons in a natural branch of the MG nerve cut at the entry into the muscle. Records obtained with both techniques indicate that, under some conditions, motor units recruited during the TVR are inhibited by sural tetani at the same time that other, previously inactive, units are excited. The MG nerve filament recordings in particular provide unambiguous evidence that the net balance of synaptic effects under these conditions is excitatory in some MG motoneurons and inhibitory in others. The methods used do not permit identification of the inhibited MG units as slow twitch, although this may be inferred from indirect evidence cited above. The main conclusion drawn from these observations is that the organization of synaptic input to a motor unit pool is a critical factor controlling the recruitment patterns exhibited by units within that pool.

1413 SYMPATHETIC NEURAL MECHANISMS UNDERLYING PARALYTIC ILEUS IN MONKEYS K Alan Kelts, Dept Neur, Standford U, Standford, CA 94305

Paralytic ileus is the cessation of gastrointestinal movements induced by laparotomy, abdominal trauma, or peritonitis. Although the splanchnic nerves apparently contain the preganglionic sympathetic fibers mediating this phenomenon, postganglionic mechanisms may involve the release of catecholamines (CA) into the circulation by the adrenal medulla or the release of CA directly on the intestinal smooth muscle via sympathetic nerves. In order to investigate these possibilities, six rhesus monkeys were immobilized in primate chairs and given 30 cc of Barospers by nasogastric tube, followed by serial abdominal x-rays (UGI). Transit times (TT) for the initial UGIs were as follows: stomach to duodenum 3.5±1.5 min, to the jejunum 5.2±4.6 min, to the cecum 29±8 min, to the descending colon 104±69 min, and to the rectum 117±98 min. Peritonitis, induced by the injection of 1.5 cc of Lugol's solution intraperitoneally, prolonged transit times, e.g., stomach to the duodenum and jejunum 76±48 min and to the cecum 245±218 min. Following these initial studies, bilateral splanchnicotomies were performed on 2 monkeys, bilateral adrenalectomies with steroid replacement on 2 monkeys, and bilateral thoracolumbar dorsal rhizotomies on 2 monkeys. Although no significant difference in TTs existed between routine UGIs pre- and post-surgery, comparison of TTs during peritonitis revealed complete abolition of ileus in 2 monkeys, one with splanchnicotomy and one with adrenalectomy. Abolition of ileus in the remaining 2 monkeys required both procedures. Dorsal rhizotomy abolished abdominal rigidity without shortening the ileus. These data suggest that both adrenal catecholamine output and direct sympathetic innervation of the bowel may operate to produce paralytic ileus.

1414 CENTRAL ACTIONS OF THE SENSORY AND MOTOR COMPONENTS OF STINGRAY SPINAL NERVES. R.B. Leonard, P. Rudomin and W.D. Willis. Marine Biomedical Inst., Galveston, Texas 77550 and Centro de Investigación del Instituto Politécnico Nacional, México 14, D.F.

The spinal nerves of many elasmobranchs are subdivided into two components which are respectively continuous with the dorsal and ventral roots of the spinal cord. The central effects of electrical stimulation of the sensory and motor components were investigated. Stingrays (Dasyatis sabina) were initially anesthetized with MS 222; after the spinal cord was sectioned at a cervical level, anesthesia was discontinued and the animals curarized and artificially ventilated. The spinal cord was exposed by laminectomy, and several pairs of spinal nerves were isolated and separated into sensory and motor components. Electrical stimuli were applied to the spinal nerve components while the spinal cord was searched with a glass microelectrode (filled with 4M NaCl; initial impedances 3-5 MΩ). Stimulation of the sensory component of a spinal nerve evoked a negative field potential which was largest in the dorsal part of the spinal gray matter. The potential reversed its polarity in the ventral horn. The onset of the potential had a short latency and was judged to be monosynaptic. Most of the potential could be produced by the large myelinated sensory fibers, although small fibers contributed to the later part of the field potential. Interneurons were sometimes encountered which discharged during the field potential. Volleys in the motor component of the spinal nerve produced a short-latency negative field potential which was largest in the ventral horn. The field inverted in the dorsal horn. It was apparently due to the antidromic invasion of motoneurons, since intracellular recordings of antidromic action potentials from motoneurons had a similar latency. (Supported by USPHS grants NS 11255 and NS 09196.)

1415 UNMYELINATED FIBERS IN RAT VENTRAL ROOTS. C.W. Maynard*, R.E. Coggeshall D.G. Emery and H. Ito*. Depts. of Anatomy and Physiology and Marine Biomedical Institute, Univ. of Texas Med. Branch, Galveston, Texas 77550.

It has become apparent that a significant population of axons in cat ventral roots are unmyelinated and sensory. It would be desirable to trace the central processes of the axons, but the cat spinal cord is so large that there are certain technical problems. It would be desirable, therefore, to work on a smaller animal, and the rat was chosen for this work. The first step in such a study is to determine the percentages of unmyelinated fibers in the ventral roots of various body segments. Three rats were anesthetized and perfused with a mixture of 1% paraformaldehyde, 1% glutaraldehyde and 0.01% picric acid in 0.1M cacodylate buffer. Ventral roots from all segments were taken and all myelinated and unmyelinated fibers from each root were counted. Ventral roots T1-L2 and L6-S1 contained approximately 30% unmyelinated axons whereas the other roots have relatively few unmyelinated axons. This distribution contrasts to that of the human and cat where all studied ventral roots have significant numbers of unmyelinated axons. The reasons for the restriction of unmyelinated axons to certain ventral roots in the rat is not clear. It may be that the unmyelinated fibers are preganglionic visceral efferents, but the L6 and S1 distribution would be unusual. Experimental work to determine the location of the cell bodies of the ventral root unmyelinated axons is underway and if the cell bodies are located in the dorsal root ganglion, then studies tracing the central projections of these fibers will begin. Supported by NIH grant NS 11255 and Training Grant DHEW 00459-15.

1416 Microcirculatory Alterations in Feline Spinal Cord following Compression Injury. Eugene D. Means, Douglas K. Anderson, Gregory B. Nicolosi* and Joanne Gaudsmith*. Research Service, Tampa V.A. Hosp. and Depts. Med. and Physiol., U.S.F. Col. Med., Tampa, Fl. 33612.

Microcirculatory changes were studied in the thoracic and lumbar segments of the feline spinal cord (SC) at various intervals up to 30 days following compression at L-2 with an 170 gm. wt. for 5 minutes. The animals were sacrificed by intra-aortic perfusion fixation using 10% neutral buffered formalin followed by a mixture of colloidal carbon (Pelikan C11/1431A), gelatin and mannitol. The SC was left in situ overnight at 4°C, removed and divided into 3 mm sections. These were labelled according to their cephlad or caudad relationship to the lesion. One-half of the sections were embedded in paraffin, cut serially at 20 μm, and stained with hematoxylin & eosin. The other sections were cut serially on a freezing microtone at 60 μm. By 8 hours post-compression there was essentially no filling in gray matter vessels. Although qualitatively estimated, a decreased filling of vessels was also apparent in white matter. These changes were most obvious at the site of the lesion & progressively diminished until normal filling was noted 3-6 mm cephlad and caudad. Decreased vessel filling appeared most marked in central gray matter of the anterior and posterior horns adjacent to the central canal. This observation may relate to occlusion of sulcal arteries which were surrounded by frank blood & which supply the central SC gray matter. Ischemic nerve cell change was evident in 1/2 hour survivals and was first noted in large motor neurons of the ventral & lateral portions of the anterior horns. Ischemic nerve cell change did not bear a specific topographic relationship to diminished vessel filling. Long term survivals showed an increased number of vessels in preserved gray & white matter.

1417 THE EFFECT OF FLUOXETINE ON SPINAL REFLEX ACTIVITY. Norbert R. Myslinski. University of Maryland, Baltimore, Md. 21201

This study investigated the effects of Fluoxetine (Lilly 110140)(F), a selective Serotonin (5-HT) reuptake inhibitor, on spinal reflex activity in the acute spinal cat. A 10 mg/kg i.v. dose of F produced no significant change in either the amplitude or latency of the monosynaptic reflex (MSR). F did potentiate the excitatory effect of 50 mg/kg of the 5-HT precursor, 5-hydroxytryptophan (5-HTP) on the MSR. This potentiation, however, was considerably less than that produced by other less specific reuptake inhibitors, such as Imipramine. The effects of both 5-HTP and F were subsequently inhibited by the 5-HT antagonist, Cinanserin. F had no significant effect on the polysynaptic reflex when used alone or after 5-HTP pretreatment. The implications of this data plus the effects of F on other evoked and spontaneous spinal activities will be discussed. (Supported in part by a grant from the PMA Foundation).

1418 AFFERENT FIBERS IN CAT VENTRAL ROOTS AS DEMONSTRATED BY AXONAL FLOW. Irving Nadelhaft and Roseanne Smorul.* VA Hosp. University Drive C, Pittsburgh, Pa. 15240, and Dept. of Neurological Surgery, University of Pittsburgh, School of Medicine, Pittsburgh, Pa. 15261

A five microliter volume of tritiated leucine (5mC/ml concentration) was injected into L7 and/or S1 dorsal root ganglion, through a bevelled micropipet. After a delay of about three hours, the dorsal and ventral roots as well as the ganglion were removed and placed in buffered formalin overnight. Following several hours of washing, each root was divided into 2 mm pieces which were solubilized and counted in a liquid scintillation counter. The uninjected contralateral sides served as controls and were treated in the same manner as the injected sides. The data from the dorsal root are consistent with a flow rate of approximately 380 mm/day. The number of counts per minute per 2 mm piece was about 300 for the dorsal root and ranged between 2% and 15% of that for the ventral root. These counts have had background of about 35 cpm subtracted from them but are not corrected for counting efficiency (about 45%). The combined average of the ratio of counts in the ventral root to counts in the dorsal root was $8.4 \pm 5.6\%$ (15 cases).

1419 PROJECTION OF SINGLE SEMITENDINOSUS IA AFFERENT FIBERS TO MOTONEURONS. S. G. Nelson and L. M. Mendell. Duke Medical Center, Durham, N.C. 27710

We have investigated the projection of single Ia afferent fibers of the hip extensor-knee flexor semitendinosus muscle (ST) to homonymous motoneurons in order to compare the divergence patterns and EPSP amplitudes to previous data for the ankle extensor triceps surae muscles (MG, LG and SOL). These studies were performed in anesthetized cats and the individual EPSPs were obtained using the spike triggered averaging technique. We found that most single afferents projected to virtually all homonymous motoneurons as in triceps surae. In one case, an afferent projected to fewer than one half of the homonymous motoneurons; similar findings have been made for some triceps surae afferents. The aggregate projection frequency was 80% (56 out of 70) which is similar to triceps. The mean EPSP amplitude was 82 μV which is smaller than the mean homonymous EPSP for MG, LG and SOL. The major difference was in the proportion of large EPSPs (>150 μV) which was smaller in the ST system than in the others. The rise times and half widths of the EPSPs in ST were similar to those in MG and LG but shorter than those for SOL motoneurons. The ST muscle is larger than either MG, LG or SOL with 2 separate nerve branches supplying distinct portions of the muscle. We have found no consistent differences in the projection frequency of afferents to motoneurons supplying the region of the muscle from which the afferent arises or to motoneurons supplying the other region. The EPSP properties (amplitude and shape) are also similar for these two types of projection. Preliminary data indicate a less extensive projection to semimembranosus motoneurons and also smaller individual EPSPs (average 29 μV, N = 8) in agreement with the smaller aggregate EPSPs evoked by ST in these motoneurons. (Supported by NS 34608, NS 08411, GM 00929).

1420 ARE DORSAL ROOT POTENTIALS GENERATED BY ACCUMULATION OF EXTRACELLULAR POTASSIUM? R.A. Nicoll, Dept. of Pharmacol. & Physiol., Univ. of Calif., San Francisco, Calif. 94143.

Recent experiments using K-selective electrodes to record changes in extracellular potassium (K_e) in the spinal cord have revived the hypothesis that the dorsal root potential (DRP) is generated by an elevation in K_e following an afferent volley. To test this hypothesis, changes in K_e in the dorsal horn region of the isolated hemisected frog spinal cord have been estimated by recording from presumed glial cells. By altering K in the Ringer, it was found that the glial cell membrane potential was determined largely by K_e. Single DR volleys resulted in a glial cell depolarization of 1.2 mV which corresponded to an increase in K_e of 0.2 mM. By altering K in the Ringer and recording from the DR with sucrose gap, it was found that 0.2 mM K would depolarize the DR by only 0.5 mV, while the DRP was 10 mV in size. K_e had to be increased by 5 mM to generate a 10 mV depolarization of the DR. The time course of the two events was also very different. The time to peak (700 ms) and 1/2 decay (4.6 s) of the glial response were much slower than those of DRPs (50 ms and 0.6 s). Tetanization of the DR resulted in a summation of the glial response which reached levels equivalent to 8 mM K_e and declined slowly (1/2 decay = 10-20 s) after stimulation. DRPs remained of constant size throughout the tetanus and subsided quickly (1/2 decay = 1-2 s) after stimulation. Tetanization of the ventral root failed to alter K_e and yet generated DRPs. In conclusion, these results suggest that accumulation of K_e plays, at most, a minor role in generating DRPs since (1) the time course of the change in K_e and the DRP is markedly different, (2) the magnitude of the increase in K_e is at least an order of magnitude too small, and (3) VR stimulation fails to alter K_e and yet generates DRPs. (Supported by the UCSF Academic Senate and a PMAF Research Starter Grant).

1421 CONVERGENCE OF GROUP I AFFERENT INPUT IN SINGLE UNITS OF DSCT. R. E. Poppele, S. Kubota* and C. K. Knox. Laboratory of Neurophysiology, Univ. of Minnesota, Minneapolis, MN 55455.

Cells projecting in the dorsal spinocerebellar tract (DSCT) that receive proprioceptive input are considered to act as relays for specific receptors in a single muscle or small group of muscles. We have used group I intensity electrical stimulation from four diverse muscle groups (gastrocnemius-soleus, anterior tibial, quadriceps and hamstring) in cat and recorded single unit activity in DSCT. Using a sensitive cross-correlation technique we have shown that most units identified as belonging to the DSCT by antidromic cerebellar stimulation respond to inputs from each muscle nerve stimulated. Of 80 units recorded, 75% responded to each muscle nerve stimulated and there was a response recorded in 84% of the total trials. Of these responses, 20% were judged to be monosynaptic. The rest had latencies greater than 5 ms and of these 59% were inhibitory responses and 41% were excitatory. There was no consistent pattern of excitation and inhibition among the muscle nerves stimulated. (Supported by USPHS Grant NS 11695.)

1422 NEURONAL PROJECTIONS INTO THE REGION OF THE SPINAL-CORD MOTOR NUCLEUS OF THE INTRINSIC PLANTAR MUSCLES IDENTIFIED BY THE HRP METHOD. Eric Proshansky* and M. David Egger. Dept. Anat., C.M.D.N.J. - Rutgers Med. Sch., Piscataway, N. J. 08854.

We have been investigating the circuitry of a cutaneous spinal reflex in the cat, elicited by stimulation of the central foot pad of the hind limb. To provide additional information about neurons projecting into the motor nucleus of the intrinsic plantar muscles, which are activated in this reflex, iontophoretic injections of horseradish peroxidase (HRP) were made into the dorsolateral ventral horn of the first sacral (S1) spinal segment. HRP-filled micropipettes (15-60 μm tip diameters, fille with 30% HRP in 0.01M saline) were used to record field potentials, initially from antidromic activation of an S1 ventral root, then during orthodromic activation of the reflex. When field potentials related to motoneuronal activation by foot-pad stimulation were maximal, HRP was conducted from the electrodes with injection currents of 4 μA for 3-5 min. Following 24 hr survival, the cats were killed, and the lumbosacra segments of their spinal cords processed by standard procedures for the demonstration of HRP. At the sites of the electrodes tips, we found HRP reaction product in deposits varying from 0.3-1.0 mm in diameter. Neurc labeled by retrogradely transported HRP were found bilaterally from the fifth lumbar to lower sacral levels. Most neurons were located ipsilaterally, many in lamina VII, with fewer in laminae VIII and IX. Many contralateral neurons were found in lamina VIII. In the dorsal horn, labeled neurons were found ipsilaterally in laminae IV-VI. The labeled dorsal-horn neurons were most often located medially, corresponding to the positions of interneurons located electrophysiologically following stimulation of the central foot pad.

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1423 EFFECT OF FETAL DECAPITATION OR PROSENCEPHALECTOMY ON THE STRUCTURAL ORGANIZATION OF THE SPINAL CORD IN THE PRIMATE. Lee T. Robertson, William A. Stotler*, Hideo Uno* Neuro. Sci. Inst. of Good Samaritan Hospt., Dept. Anat., Univ. Ore. Health Sci. Cent., Portland, Oregon; Dept. Path., Ore. Reg. Primate Center, Beaverton, Oregon.

The structural organization and fiber distribution of the spinal cord were examined in 11 fetal rhesus monkeys sacrificed at 143-147 days gestation (full term=168±4 days). Approximately 70 days prior to sacrifice 6 of the fetuses were decapitated and 3 fetuses underwent prosencephalectomies, which also damaged the rostral mesencephalon. Two fetuses developed normally. Adjacent serial sections were stained with Stotler silver, Weigert and Holzer methods.

The spinal cord diameter at all levels was approximately 25% smaller after prosencephalectomy and almost 35% smaller after decapitation than the normal cord. In both, the reduction in size was due to fewer fibers, mainly within the ventrolateral funiculus, with only slight decreases in the ventromedial and dorsal funiculi. In the decapitated animals, the fasciculus proprius could be distinguished from the larger axons composing the dorsal and ventral spinocerebellar tracts. There was no evidence of any demyelination, necrosis, or increased cellular gliosis within the white or gray matter in either condition. The organization of the dorsal horn was not affected; likewise, the size, shape, and axis orientation of large motoneurons in the ventral horn were the same for normal and experimental animals. In the ventral horn, however, the internal fibrous matrix was considerably reduced in the prosencephalectomy monkeys and even more so in the decapitated group. Consequently, some connecting and transversing fibers were clearly delineated, especially those from the fasciculus proprius and the spinothalamic tract.

1424 PRESYNAPTIC CONTROL OF INFORMATION TRANSMISSION IN THE STINGRAY SPINAL CORD. P. Rudomin, R.B. Leonard and W.D. Willis. Marine Biomedical Inst., Galveston, Texas 77550 and Centro de Investigación del Instituto Politécnico Nacional, México 14, D.F.

Most of our knowledge concerning presynaptic control of synaptic transmission in the spinal cord is derived from studies in mammals and amphibia. Here we present evidence for primary afferent depolarization (PAD) and presynaptic inhibition in elasmobranchs. Stingrays (Dasyatis sabina) were initially anesthetized with MS 222; after the spinal cord was sectioned at a cervical level, anesthesia was discontinued, and the animals were curarized and artifically ventilated. The spinal cord was exposed, and several pairs of spinal nerves were isolated and separated into sensory and motor components. The negative field potential recorded from the dorsal horn in response to stimulation of the sensory portion of a spinal nerve was depressed when an adjacent sensory nerve was stimulated. The depression was seen with conditioning-test intervals of 10-150 ms, with a maximum at 20-30 ms. The excitability of large myelinated afferent fibers was tested, using Wall's technique. A metal microelectrode was introduced into the dorsal horn to stimulate afferent fibers. The antidromic response was recorded in the sensory portion of the spinal nerve. Conditioning volleys in an adjacent sensory nerve produced an increased excitability of the afferents; the excitability increase had a time course similar to that described for the depression of the field potential. Reflex discharges were recorded from the motor component of a spinal nerve. Conditioning volleys in sensory fibers of an adjacent segment did not inhibit such reflexes, but instead produced a long-lasting facilitation. However, most of the reflex discharge could be attributed to the activation of small myelinated fibers, rather than of the large fibers. (Supported by USPHS grants NS 09196 and NS 11255.)

1425 AN ULTRASTRUCTURAL STUDY OF THE BRACHIAL GLYCOGEN BODY IN THE SPINAL CORD OF THE DOMESTIC CHICKEN. Frances M. Sansone. Dept. of Anat. Sci., State University of New York at Buffalo, Buffalo, N.Y. 14214.

Birds, domestic and wild, are unique in that they have a glycogen storage deposit, the glycogen body, within their lumbosacral spinal cord (Watterson, J. Morph. '49). Recently, Sansone and Lebeda (J. Morph. '76) showed that glycogen-rich cells similar to those that form the glycogen body are found surrounding the central canal in the brachial plexus region of the domestic chicken spinal cord. In a more extensive study, Sansone (Anat. Rec. '76) demonstrated a glycogen-rich area surrounding the central canal at all levels of the spinal cord and closed medulla. When the central canal opens into the fourth ventricle, the glycogen-rich tissue is found in the midline floor of the ventricle until the level of the oculomotor complex, where it ends.

In toluidine blue stained thick sections, the brachial glycogen body appears as a bulbous accumulation of vacuolated material immediately surrounding the ependymal cells of the central canal and extending into the posterior median septum. At the ultrastructural level, the vacuolated area is seen to consist of numerous astroglial cells whose cytoplasm is interrupted by many spaces which contain a few or many beta type granules of glycogen and bundles of gliofilaments interspersed with glycogen granules of varying sizes. Astrocytic perinuclear cytoplasm is filled with an enlarged granular endoplasmic reticulum and mitochondria. The ependymal cells have microvilli and cilia on their apical surfaces and desmosomal-like structures on their lateral, luminal margins. Their apical cytoplasm contains some large glycogen particles. Although the functional significance of the glycogen body is unknown, its morphology supports the hypothesis that it is a reservoir of readily available glucose for the cerebrospinal fluid. (Supported by a UBF Grant).

1426 TESTING FOR REGENERATION IN RAT SPINAL CORD WITH A SCINTILLATION COUNTING PROCEDURE. Lawrence M. Shuer*, Earl R. Feringa, and H. Lee Vahlsing*. Dept. Neurology and Pathology, Ann Arbor VA Hospital and University of Michigan Medical School, Ann Arbor, Michigan 48105.

A controlled experiment designed to study regeneration of the spinal cord in 40 immunosuppressed rats and 14 controls after complete transection included electrophysiologic and radiochemical methods for demonstrating axonal continuity. Three treatment groups were studied (induced tolerance to central nervous system antigens, 75 mg/kg cyclophosphamide at 1 hour, or 75 mg/kg cyclophosphamide at 24 hours post transection). Twenty-one days prior to electrophysiologic evaluation each rat was injected with 100 μc. tritiated proline in the motor cortex. The isotope should be transported by axoplasmic flow down motor axons.

Each animal was tested for electrophysiologic evidence of regeneration by stimulation of the cervical corticospinal tract while recording from the ipsilateral sciatic nerve. No difference was found between treatment and control groups.

Samples of spinal cord were removed and prepared for scintillation counting. Disintegrations per minute per unit length were determined both above and below the site of spinal cord transection. Statistical analysis of the proportion of radioactivity found below the site of transection as a function of that found at higher levels demonstrated that the group of 13 rats who received 75 mg/kg cyclophosphamide one day after transection had significantly higher amounts of tritiated proline below the site of transection.

1427 MONOSYNAPTIC INPUTS TO VENTRAL HORN INTERNEURONS FROM THE LATERAL VESTIBULAR NUCLEUS AND THE MEDIAL LONGITUDINAL FASCICULUS. Robert D. Skinner. Dept. Anatomy, Univ. Ark. College of Medicine, Little Rock, AR 72201.

Silver degeneration studies have demonstrated almost identical regions of termination in the medial ventral horn for the lateral vestibulospinal and the pontine reticulospinal tracts, the latter of which has axons descending in the MLF and adjacent reticular formation (Petras, Brain Res., 6:275, 1967). In order to study these terminations electrophysiologically, neurons in the medial ventral horn of segments L_6 and L_7 were examined for separate or convergent input from these two sources, and maps of the field potentials were made. In cats anesthetized with α-chloralose (40 mg/kg) and urethane (400 mg/kg), the lateral vestibular nucleus (LVN) and the MLF (5-6 mm rostral to the obex) were stimulated in each experiment with tungsten electrodes using negative pulses of current (0.2 msec and 100 μA or less). A lesion at the L_1 level left only the ipsilateral ventral quadrant intact.

Intracellular recording was done with 2M KCitrate or 2.7M KCl micropipettes in 120 non-motoneuronal neurons. Sixty-five of these (54.2%) had monosynaptic (MS) EPSPs. The distribution of the MS EPSPs according to source was: 29 from LVN only, 19 from MLF only, and 17 having MS EPSPs from both. The segmental latencies of the EPSPs from the axonal volleys were 0.68 ± 0.20 S.D. msec for LVN, and 0.77 ± 0.12 S.D. msec for MLF. Disynaptic IPSPs and EPSPs also were found. Most of the neurons were in lamina VIII, some in VII, and a few in VI; none were in IX. The location of the neurons is in good agreement with the terminals seen in silver degeneration studies of others and the field potentials evoked by stimulation of the two tracts in these experiments.

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1428 LEVELS OF GABA AND GLYCINE IN CANINE LUMBAR SPINAL CORD DURING THE DEVELOPMENT OF SPINAL SPASTICITY. J.E. Smith, P.V. Hall*, A.R. Jones*, D.L. Campbell* and M.H. Aprison. Depts. of Neurosurgery, Psychiatry and The Inst. of Psychiat. Res., Indiana Univ. Med. Ctr., Indianapolis, IN. 46202.

Spinal spasticity is a clinical condition that gradually develops long after descending pathways have ceased to function. Recently, spinal spasticity has been demonstrated to be accompanied by a decrement in segmental inhibition at presynaptic, postsynaptic and recurrent inhibitory sites. We have studied changes in two segmental inhibitory neurotransmitters [GABA and glycine (Gly)] in lumbar cord during the development of spinal spasticity. Mid-thoracic cord transections were performed in adult female dogs which were then sacrificed at one (N=7), three (N=7), eight (N=7) and twelve (N=4) weeks post-transection. The lumbar enlargement of the cords was removed and rapidly frozen in liquid nitrogen. The L_2 and L_3 segments were dissected at -20°C into dorsal grey (DG) ventral grey (VG), dorsal medial white (DMW), dorsal lateral white (DLW), ventral lateral white (VLW), and ventral medial white (VMW). Gly and GABA were extracted into TCA and assayed by GLC as their respective DNP-methyl ester. Compared to unoperated controls (N=10), GABA levels fell to 76% in VG at 3 weeks, and then increased to 137% at 12 weeks. In DG little change occurred in the first 3 weeks and then GABA levels increased to 169% at 12 weeks. Gly did not change in DG but decreased significantly in VG at 8 (to 65%) and 12 weeks (to 73%). There did not appear to be significant changes in GABA levels in the four different areas of white matter. However, Gly was significantly decreased at 12 weeks in VLW (to 49%), a finding consistent with neuroanatomical data. These changes in GABA and Gly are consistent with the previously reported decrement in presynaptic, postsynaptic and recurrent inhibition observed in this clinical condition. (Supported in part by PHS Grant MH 03225-17 and the L. Freeman Memorial Fund).

1429 FUNCTIONAL ORGANIZATION OF THE DORSAL SPINAL GRAY MATTER IN CAT. Daniel Tapper and Zsuzsanna Wiesenfeld. Dept. Physical Biol and Section of Physiology, N.Y. State Coll. Vet. Med., Cornell U., Ithaca, N.Y. 14853

Temporal, spatial and feedback interactions between lamina IV and V neuron of the first sacral segment were studied by introducing one or a pair of action potentials into these networks via afferent fibers emerging from Type I skin receptors, Haarscheiben(Hs). Poststimulus time histograms of central unit activity were derived in response to sequences of 100 stimuli applied not less than once per three seconds. When conditioning-test pairs were introduced via a single afferent axon (temporal interactions), a characteristic response curve was observed consisting of an early increase in discharge number lasting up to 30 ms. followed by a reduction in response lasting up to 500 ms. When the response consisted of early and late components, occasionally only the late portion was influenced; rarely, only inhibition or facilitation was observed. Similar response curves were found when the stimulus pairs were applied to different Hs (spatial interactions). No systematic relationship of distance between the two Hs stimulated and extent of interaction has yet been established. In cases where the central cell had habituated to stimulation of a given Hs, the stimulus was often still effective in interacting with that applied to another Hs, thus indicating that the habituating portion of the network can be separate from other portions. When single impulses are introduced at specified times in relationship to the central cell's ongoing discharge (feedback interaction) a variety of response curves were generated. These often differ from those derived from temporal and spatial interaction studies indicating that feedback interactions may involve additional portions of the central network. From these data we can derive modules of network function involving groups of neurons, information of importance for modelling the network as a whole (Supported by USPHS Grant NS07505)

1430 ANATOMICAL ORGANIZATION OF THE CAT PHRENIC NUCLEUS. Charles L. Webber, Jr., Robert D. Wurster and Jin Mo Chung. Department of Physiology, Loyola University Stritch School of Medicine, Maywood, IL. 60153.

The functional characteristics of phrenic motoneurons may in part depend upon the anatomical organization of the phrenic nucleus. However, adequate detailed description of the distribution and spacing of cat phrenic cell bodies is lacking due to inherent problems associated with the standard marking techniques utilized. To overcome these limitations, the retrograde transport of horseradish peroxidase (HRP) was used to map the cat phrenic nucleus. In pentobarbital anesthetized cats that had one phrenic nerve severed, the entire diaphragm was covered with 20% HRP by multiple injections. Following 48 to 72 hours survival time the cervical cord from C4 to C7 and the intact phrenic nerve were removed for histological processing. Stained phrenic cells were grouped discretely in the middle of the ventral horn with population borders extending from lower C4 to middle C6. The number of labeled cells was in approximate agreement with direct phrenic fiber counts. All identified neurons were ipsilateral to the intact phrenic nerve implying that the crossed-phrenic phenomenon is probably mediated by internuncial fibers that cross in the cord. The cell bodies formed a very homogeneous nucleus that was often organized into tight clusters with inter-phrenic distances less than 30 μm. This dramatic dense cell packing may suggest a high probability of interaction among phrenic motoneurons important for the time locking of these cells during inspiration. Thus the synchronization of phrenic burst discharges may be enhanced by overlapping of dendritic fields, ephaptic coupling, and the accumulation of extracellular potassium ions. (Supported by NIH Grant HL 08682.)

1431 CHOLINE ACETYLTRANSFERASE ACTIVITY IN LARGE VENTRAL SPINAL NEURONS. David E. Weil and D.L. McIlwain. Dept. Physiol. UNC Sch. of Med., Chapel Hill, NC 27514

Bulk fractions of large ventral spinal neurons, most of which may be alpha motoneurons, can be isolated from bovine spinal cord (Capps-Covey and McIlwain, J. Neurochem. 25, 517, 1975). On the average, each purifie cell body synthesizes 2.0 $\pm$ 0.6 (S.D.) pmoles of acetylcholine per hour at optimal enzyme and substrate concentrations. Choline acetyltransferas (ChAc) activity, determined by the method of Fonnum (J. Neurochem. 24, 407, 1975), does not appear to decline during the post-mortem period preceding cell isolation. Greater than 90% of the activity is soluble and readily released from the dissociated neurons at pH 7.4 in 50mM Na phosphate buffer - 0.32M sucrose. On the other hand, loss of ChAc from the cell bodies during their isolation is minimized if the enzyme is kept particulate throughout the procedure by use of low ionic strength media at pH 5.0. Maximal ChAc activity in the isolated cells is 60-80 times less than that of acetylcholinesterase, while the specific activities of the two enzymes show almost equivalent increases as one proceeds from ventral grey matter to isolated neurons. Each enzyme probably represents less than 0.05% of the total protein in the isolated neuronal cell bodies.

1432 RESPONSE PROPERTIES OF DORSAL HORN INTERNEURONS TO A SINGLE ACTION POTENTIAL IN A CUTANEOUS NERVE. Zsuzsanna Wiesenfeld and Daniel Tapper, Dept. Physical Biology and Section of Physiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

The organization of the dorsal spinal gray neuronal network involving cells of laminal IV and V of the first sacral segment of cat was studied in low spinal animals anesthetized with urethane. These cells have convergent input from various types of cutaneous afferents, including Haarscheiben. We monitored the spontaneous activity of single cells and studied their response to a single action potential evoked in a cutaneous afferent of the posterior femoral cutaneous nerve by electrical stimulation of a Haarscheiben.The stimulus rate was once per three seconds. The kinds of responses, their latency, probability of firing, and stability over time were studied. Monosynaptic, multisynaptic, and mixed responses were observed. Occasionally a postresponse inhibition of ongoing discharge occurred. Some of the multisynaptic responses habituated during a 100-stimulus sequence. Most responses were stable and repeatable over many hours, as judged by the form of the poststimulus time histogram. For a number of cells the response properties of each Haarscheiben in the receptive field were examined. In some cells only one response type could be found and in others the receptive field was a mosaic of response types with various firing probabilities. No orderly arrangement of receptor sites could be discerned within the receptive fields. It was not uncommon to find groups of Haarscheiben within the receptive field that, when stimulated, did not evoke a response in the interneuron. The axons of these cells of the dorsal horn do not project rostrally in the spinal cord many segments because they could not be driven antidromically by stimulation of various ascending pathways. (Supported by USPH Grant NS07505)

1433 Cells of Origin of Propriospinal Connections to Cat Lumbosacral Gray Matter as Determined with Horseradish Peroxidase. Robert P. Yezierski, James L. Culberson, and Paul B. Brown. Dept. of Physiology & Biophysics and Dept. of Anatomy, West Virginia University Medical Center, Morgantown, WV 26506.

Large horseradish peroxidase (HRP) deposits in cat lumbosacral cord were made using pressure injections to determine gross patterns of connectivities to the hindlimb region, preliminary to small iontophoretic injections for more specific connectivity studies. HRP was injected in volumes of 3-10µl in dorsoventral tracks in gray matter of L_3-S_1 segments of cats, injecting through a 15-50µ diameter pipette during withdrawal from a penetration into the ventral horn. HRP deposits were found in white and gray of dorsal and ventral quadrants, usually over complete mediolateral extent of gray matter, often extending into contralateral gray. Anteroposterior extent ranged from 0.5 to 2 segments, after survival times of 24-50 hours. Cells with HRP granules were seen as far rostral as C_2 and as far caudal as S_1. At C_2 and C_3, sparse filled cells were seen in lamina VI and ventral horn. In C_4, sparse cells were seen in laminae IV-VI and ventral horn. Below C_4, cells with HRP became increasingly common, with cells in substantia gelatinosa appearing at mid-lumbar levels. Within 2-3 segments rostral and 1-2 segments caudal to the injection, filled cells were common in all laminae. Our results indicate substantia gelantinosa cells projecting to hindlimb region are themselves in the hindlimb region. Laminae IV and V have a greater rostrocaudal spread of projection cells; lamina VI and ventral horn have the most extensive rostrocaudal spread of projection cells, particularly from more rostral segments. Further studies are in progress to determine cells of origin of projections to circumscribed areas of the hindlimb map.

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Synaptic Transmission

1434 BIOCHEMICAL EVIDENCE FOR THE Ca^{++}-DEPENDENT VESICULAR RELEASE OF ACh. Paul T. Carroll * and Alan M. Goldberg. The Johns Hopkins School of Hygiene and Public Health, Baltimore, Md. 21205

To determine the site of the Ca^{++}-dependent release of ACh, we examined the differential effects of Ca^{++} omission and Mg^{++} elevation on the K^+ induced release and subcellular localization of newly synthesized (nACh) and total ACh (tACh). Initially, minces of mouse brain were incubated in Krebs medium for 4 min with 100uM ^{14}C choline (^{14}C-Ch) and then transferred to either 35mM K^+ Krebs or Ca^{++} free 35mM K^+ Krebs for 4 min. K^+ releases both n and tACh into the media and decreases the levels of each in the cytoplasmic (2.8 to 1.5 and 12.6 to 6.9 nmol/g, respectively) and vesicular fractions (1.5 to 0.9 and 10.1 to 5.7 nmol/g). Omission of Ca^{++} significantly reduces the K^+-induced release of n & tACh from the tissue into the media from 5.0 to 2.5 and 11.7 to 5.3 nmol/g, respectively, and blocks the release of n & tACh from the vesicular fraction. However, Ca^{++} omission blocks the release of only nACh from the cytoplasm. To observe this latter effect, an AChE inhibitor was required during or prior to tissue homogenization. An AChE inhibitor was not required to show that Ca^{++} omission blocks the release of n & tACh from the vesicular fraction. Hemicholinium (100uM) significantly reduces the Ca^{++}-dependent release of tACh (6.2 to 2.5 nmol/g) without inhibiting the Ca^{++}-independent release of tACh. Omission of Ca^{++} during K^+-induced ACh release in the presence of hemicholinium blocks the release of n & tACh from the vesicular fraction without changing either in the cytoplasmic fraction. This result suggested that the uptake of extracellular choline during K^+ depolarization might account for some of the Ca^{++}-dependent ACh released. We thus tested the possibility that Ca^{++} omission might block the movement of ACh, formed from extracellular choline, through the cytoplasmic and vesicular fractions. Minces of mouse brain were incubated with 100uM ^{14}C Ch for 4 min in one of the following Krebs media: normal, 35mM K^+, Ca^{++} free 35mM K^+, and 16mM Mg^{++} 35mM K^+. The results indicate that Ca^{++} omission and Mg^{++} elevation significantly decrease the K^+ induced release of n & tACh into the media. Omission of Ca^{++} blocks the K^+-induced movement of nACh through both the cytoplasmic and vesicular fractions since the levels are similar to those found following incubation in normal Krebs and significantly higher than those found in minces incubated in 35mM K^+ Krebs. Elevated Mg^{++} blocks the release of n & tACh from only the vesicular fraction. The possibility was then tested that the movement of nACh into the vesicular fraction might be facilitated by the Ca^{++}-dependent release of preformed ACh out of the vesicular fraction. Minces were first incubated in either 35mM K^+ Krebs or Ca^{++} free 35mM K^+ Krebs for 4 min and then transferred to normal Krebs with 100uM ^{14}C-Ch and incubated 4 min. The results show that the ratio of n to tACh increases 60% in the vesicular fraction but not in the cytoplasmic fraction of minces incubated in 35mM K^+ Krebs relative to those incubated in Ca^{++} free 35mM K^+ Krebs. When the minces were subsequently transferred to a 35mM K^+ Krebs media, the ratio of n to tACh released from the minces first incubated in K^+ was 68% greater than those incubated in Ca^{++} free K^+ but the total amounts of ACh released from both preparations were identical. In summary, the results indicate that the synthesis of the Ca^{++}-dependent fraction of releasable ACh may occur in a cytoplasmic pool which fails to mix with preformed cytoplasmic ACh since the K^+ induced release of the latter is not Ca^{++}-dependent. The movement of ACh into the vesicle appears to be facilitated by the emptying of preformed ACh out of the vesicle during K^+-induced ACh release. Supported in part by NIEHS grants 00034 and 00454.

1435 EVIDENCE THAT SINGLE VERTEBRATE CNS NEURONS CAN MEDIATE BOTH ELECTRICAL AND CHEMICAL INHIBITIONS. Donald S. Faber and Henri Korn*. Res. Inst. on Alcoholism and Dept. Physiol., SUNY at Buffalo, Buffalo, NY 14203.

The hypothesis that the same medullary interneurons which generate electrical inhibition of the goldfish Mauthner cell (M-cell) also mediate postsynaptic chemical inhibition of that cell has been confirmed with simultaneous intracellular recordings from the pre- and postsynaptic neurons. Interneurons mediating electrical inhibition were identified by a characteristic "passive hyperpolarizing potential" (PHP) recorded during an M-cell antidromic spike. Since the PHP neurons belong to the M-cell's recurrent collateral network (Korn and Faber, J. Neurophysiol; 38:452, 1975), unitary IPSPs produced by impulses in these neurons were compared with the full collateral inhibition which follows M-cell antidromic invasion. Three experimental approaches were used to demonstrate a unitary IPSP following a directly evoked spike in one presynaptic neuron: 1) at M-cell resting potential no IPSP was observed but its underlying conductance change was demonstrated as a 1 to 7% reduction (m=2.8%, n=13) in the cell's antidromic spike height when a spinal stimulus was preceded by the presynaptic impulse; 2) small unitary hyperpolarizing IPSPs were recorded during depolarization of the M-cell with transmembrane currents applied through the recording electrode; and 3) after intracellular Cl^- injections which converted the recurrent collateral IPSP to a depolarizing response, stable depolarizing unitary IPSPs which could be studied in detail were recorded. Unitary IPSP latency was monosynaptic, ranging from 0.3 to 0.78 msec (m=0.51 msec, SD=0.11, n=33), and mean IPSP amplitude ranged widely from 0.13mV to 8.7mV (m=0.89mV, SD=1.27, n=39), with the larger values being encountered infrequently. Average peak time and half decay time were 0.89 and 3.52 msec, respectively, and the time course of the conductance change paralleled that of the IPSP.

Finally, evidence that one PHP cell could evoke both electrical and chemical inhibition of the M-cell was obtained with successive intra- and extracellular recordings of the M-cell responses to a presynaptic spike; a monophasic extrinsic hyperpolarizing potential (EHP), which is the sign of the electrical inhibition, was recorded extracellularly, and a unitary IPSP was then obtained intracellularly. Electrotonic coupling between PHP neurons could not account for these observations; in 32 recorded pairs no such coupling was observed. The model for one cell generating both inhibitions assumes that failure of active spike propagation in the processes of these interneurons projecting to the M-cell's axon hillock and soma underlies generation of the electrical component, and the passively conducted terminal depolarization is above the threshold for inhibitory transmitter release. (Supported in part by NIH Grant NS-12132-02).

1436 MECHANISM OF THE SYNAPTIC ACTION OF BOTULINUM TOXIN. Ing Kao*, Daniel B. Drachman and Donald L. Price. Dept. Neurol., and Pathology, Johns Hopkins Med. School, Baltimore, Md. 21205

Botulinum toxin (BOT) prevents the release of acetylcholine (ACh) from cholinergic nerve endings. The precise mechanism of its action is unknown. In this study, two pharmacologic probes, calcium ionophores and black widow spider venom (BWSV), were used to try to determine the way BOT interferes with the synaptic release of ACh. The results suggest that BOT acts by blocking the exocytosis of synaptic vesicles at the releasing sites (active zones) opposite to postjunctional folds.

End plate potentials (epps) and miniature epps (mepps) were monitored in vitro in phrenic nerve-diaphragm preparations from Swiss mice. BOT (0.1 ug/ml) totally blocked epps and maximally reduced mepp frequency and amplitude in about 5 hours. In fully botulinized preparations, calcium ionophores (either X-537A or A23187 at 25 uM/l) did not elicit any increase in mepp frequency. In control preparations X-537 caused a 5 fold calcium dependent increase in mepp frequency. A-23187 had a similar but less consistent action. This suggests that BOT does not act by interfering with calcium influx into the synaptic terminals.

In both control and fully botulinized preparations BWSV (1 gland homogenate per 2 ml) caused a massive barrage of mepps which was independent of the presence of external Ca^{++}. This result indicates that the synaptic vesicles in botulinized terminals are not devoid of ACh. Electronmicrographs of BWSV-treated control preparations showed swollen nerve terminals virtually depleted of all synaptic vesicles. In preparations first blocked by BOT and then treated with BWSV the synaptic terminals were also enlarged and almost empty. However, they showed clusters of vesicles in close proximity to the nerve terminal membrane at active zones. This observation can be interpreted to suggest that BOT may interact with the releasing site in such a manner that exocytosis of vesicles at the active zones is blocked.

On the basis of these findings, it is suggested that BOT acts beyond the step of Ca^{++} influx, that it does not deplete synaptic vesicles of ACh, and that its most likely action is interference with exocytosis at the normal releasing sites.

1437 SODIUM CHANGES: ACCOUNT FOR ELECTROLYTE SHIFTS OF SPREADING DEPRESSION. R.P. Kraig* & C. Nicholson. (SPON: A.L. Benton) Div. of Neurobiology, University of Iowa, Oakdale, Ia., 52319

Sodium movement out of the extracellular space during spreading depression (SD) has long been implied in the glutamate hypothesis of van Harreveld. Implication for its involvement comes from tissue impedance increases and histological evidences of a decrease in extracellular space and cellular accumulation of chloride which occur during SD. Direct support comes from our past measurement of a large decrease in $[Cl^-]_o$ and increase in $[K^+]_o$ during SD in catfish cerebellum. If electroneutrality is maintained in SD, these large ion shifts suggest: a) activity coefficients change; b) some unknown anion substitutes for $[Cl^-]_o$; or c) $[Na^+]_o$ falls along with $[Cl^-]_o$ creating a significant anisosmolal condition.

To test these alternatives we simultaneously measured $[Na^+]_o$, $[K^+]_o$, and $[Cl^-]_o$ during SD in the cerebellar molecular layer of *Corydoras aneus*. Measuring two ions per SD and using micropipettes containing Na, K, or Cl ion selective exchangers (10% w/w solution of monensin in nitrobenzene, Corning 477317, or 477315 respectively) reveals normal extracellular electrolyte concentrations of 149mM Na^+, 2mM K^+, and 137mM Cl^-. At the peak of similar SD's these values changed to around 57mM Na^+, 35mM K^+, and 47mM Cl^-.

This massive shift in $[Na^+]_o$ has not been recognized before. It can account for nearly all of the potassium and chloride shifts of SD. These electrolyte shifts also closely parallel recent (100-300%) extracellular impedance changes recorded during SD. Furthermore, such changes in $[Na^+]_o$ almost certainly have a profound influence on synaptic function and transmitter metabolism and transport which may be pertinent in the mechanism of SD.

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1438 EXTRACELLULAR POTASSIUM CHANGES ASSOCIATED WITH THE CONTROL OF RETINOTECTAL SYNAPTIC TRANSMISSION IN BUFO MARINUS.
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We have investigated the role of potassium ion flux as a potential mechanism for modulating transmission of visual information in the optic tectum of the toad Bufo Marinus using ion-specific microelectrodes. The mean resting extracellular potassium concentration was 1.44±0.27 mM. By varying stimulus rate, it was possible to reach a steady-state at which apparent rates of potassium release and reuptake were balanced. A comparison of the kinetics of potassium efflux for animals maintained at 4°C and 24°C yielded a Q_{10} of 1.4. This corresponds to that expected for a passive diffusion rather than an active pumping mechanism. Stimulation of the optic nerve produced a local efflux of potassium which increased logarithmically with increasing frequency, duration and amplitude of stimulation. For brief (less than 3 sec.) periods of tetanization, K^+ efflux rose rapidly and continued to rise and then fall over a prolonged time course whcih was temperature dependent, lasting longer than 30 sec. at 4°C compared to approximately half that value at 24°C. The maximum potassium accumulation following a one second tetanus consisting of 25 stimuli was 0.239±0.029 mM in animals cooled to 4°C and 0.540±0.065 mM in toads at 24°C. The change in potassium equilibrium potential produced in a uniform population of axons by repeated activity is given by:

$$\Delta V_k = 58 \log \frac{K_o + CK_i[1 - (1 - f)^n]}{K_i (1 - f)^n}$$

where C is the ratio of intracellular to extracellular space, f is the fractional loss of internal K^+ per impulse, and n is the number of impulses. This equation was derived to calculate the amount of depolarization of retinal terminals and the fractional loss of potassium per impulse from measurements made following optic nerve stimulation. The fractional loss of internal K^+ per impulse is inversely related to fiber diameter, which calculations based on K^+ flux showed to be in the range of 0.1 to 1 microns, in close agreement with the values measured anatomically and calculated from conduction velocity measurements. Extracellular volume was similarly estimated from potassium flux measurements, and ranged between 1 to 5%. Spatial location of K^+ flux following optic nerve stimulation was performed by calculating first (gradient) and second (divergence) spatial derivatives from laminar K^+ fluxes recorded at increments of 50 microns beneath the surface. Maximum efflux occured in the superficial optic layer from presynaptic retinal axons. A decrease in amplitude and increase in latency of afferents to the tectum followed the same course as the buildup of K^+. A related set of experiments using antidromic testing has shown long duration changes in the excitability of retinal terminals which parallel increased release of K^+. These results suggest that changes in extracellular K^+ induced by retinal activity might exert a significant presynaptic control over the transmission of visual information.

1439 CATECHOLAMINE RELEASE FROM ADRENAL SECRETORY VESICLES: REGULATORY ROLE OF PERMEANT ANIONS. CHRISTOPHER J. PAZOLES* AND HARVEY B. POLLARD. NIH. Bethesda, Md. 20014.

Many hormones and neurotransmitters are stored in secretory vesicles and are released from cells by the process of exocytosis. Isolated secretory vesicles (chromaffin granules) from bovine adrenal medulla can be induced to release catecholamines when exposed to ATP, divalent cations and high levels of chloride. This reaction is of interest because it may represent molecular events associated with exocytosis. ATP and magnesium have been found to regulate the transmembrane potential of isolated chromaffin granules (Pollard, et al. 1976 J. Biol. Chem., in press), and to function as a substrate for granule adenylate cyclase during catecholamine release (Hoffman, et al. 1976 J. Supramol. Struct. 4:181-184; Zinder et al. 1976 J. Biol. Chem. 251:2179-2181). By contrast, there is little information available concerning the role of chloride in the release reaction. Release depends on chloride in a positively cooperative fashion with a threshold of 20-30mM. Other permeant anions, such as thiocyanate, can replace chloride, while relatively impermeant anions such as isethionate ($HO-CH_2-CH_2-SO^-_3$), phosphate and sulfate cannot (Hoffman, et al. 1976 Arch. Biochem. Biophys., in press).

We have investigated the role of chloride in the release reaction by studying the effects of the impermeant anion, isethionate, on chloride activation. Isethionate was found to suppress baseline catecholamine release at concentrations as low as 5mM. More significantly, isethionate also blocked Cl-activated, ATP-dependent release in a dose-dependent fashion ($K_i \sim 35$mM). Kinetic analyses revealed that the inhibitory effect of isethionate on release was competitive with chloride. Isethionate did not alter the positively cooperative nature of chloride activation (see Table).

These results suggest that isethionate and chloride interact at a common site, possibly an anion channel. If so, anion permeation may be required for release to occur. The role of ATP in release may be to depolarize the secretory vesicle membrane and gate the entry of permeant anions such as chloride. Water influx accompanying this osmotic imbalance could be the driving force for release from secretory vesicles. We suggest that an analogous event may be ultimately responsible for release by exocytosis.

Isethionate (mM)	n_H	K_i^{Iseth} (mM)	V_{max}^{a}	$K_{1/2}^{Cl^-}$ (mM)
0	2.79	--	7.1	69.2
10	2.77	57.3	7.1	81.2
20	2.70	37.7	7.1	105.9
30	2.61	33.1	7.1	131.8

a. % catecholamine released/minute

1440 INCREASED NUMBERS OF NORADRENERGIC VESICLES AND EXOCYTOTIC PROFILES IN NERVES OF HUMAN VEINS AFTER FIELD STIMULATION. Åsa Thureson-Klein, Richard L. Klein and Lennart Stjärne*. Dept. Pharmacol. Toxicol., Univ. Miss. Med. Ctr., Jackson, MS 39216 and Dept. Physiol., Karolinska Inst., Stockholm, Sweden S-104 01.

Biopsy specimens of omental veins ca 1-1.5 mm external diameter were obtained during anesthesia from female patients undergoing routine abdominal surgery. As controls, small portions of tissue were placed in fixative within 10-30 sec after excision and others were superfused in oxygenated tyrode's medium, but not subjected to additional experimental procedures prior to fixation. Experimental veins were similarly superfused and noradrenaline release and tension development were induced by field stimulation (1,2) prior to fixation and embedding.

Omental veins had a rich supply of sympathetic nerves which penetrated the tunica media to mingle among bundles of smooth muscle cells with terminal portions generally located 50-500 nm from the sarcolemmae (3). The number of vesicles and the ratio between large dense core vesicles and small 'synaptic' vesicles varied greatly. In the control veins the average content was 30.8 ± 2.2% (SEM) large and 68 ± 4.0% small vesicles per terminal (n = 294 terminals). This meant that the large dense core vesicles comprised ∿ 75% of the total vesicle content volume in the average varisosity. Only a few omega-shaped 'coated' vesicle invaginations of the neurolemma, indicative of exocytosis, were observed. In all cases a large dense core vesicle was implicated.

Nerve terminals in controls which were superfused but not field stimulated contained statistically significant increased numbers of both small and large dense core vesicles, but the relative percentages were unchanged, 30.6 ± 2.3% large vesicles (n = 287 terminals). There was no significant increase in exocytotic profiles.

After field stimulation there was a highly significant increase in the number of small vesicles. Although the number of large dense core vesicles also increased, it was proportionately less, therefore, their percentage decreased to 25.1 ± 1.4 of the total vesicle population (n = 396 terminals). Many of the large vesicles were swollen and contained a more granulated matrix compared to the homogeneous content of controls. The number of exocytotic coated vesicle invaginations increased significantly, which implied that many large dense core vesicles had fused with the neurolemma to expel their contents. In some the extruded matrix material (core) was visualized, presumably just prior to dissolution.

The increased numbers of large dense core and small vesicles in incubated controls compared to those veins fixed immediately after excision was interpreted to result from continued axoplasmic transport during the period of experimentation. In addition, the field stimulation-induced increase in small vesicle numbers suggested that local formation had occurred. This could reflect a direct response to reuptake released transmitter.

(1) Stjärne, L. and J. Brundin. Acta physiol. scand. 94: 112-127, 1975.
(2) ibid, 95: 89-94, 1975.
(3) Thureson-Klein, Å., L. Stjärne and J. Brundin. Neuroscience 1: 1976 (in press).

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1441 MODULATION OF CHEMICAL POSTSYNAPTIC EFFICACY BY ELECTRICAL PRESYNAPTIC TRANSMISSION IN APLYSIA. Rafiq Waziri, Department of Psychiatry, University of Iowa Hospitals and Clinics, Iowa City, Iowa 52242

The well known large interneuron L_{10} in the abdominal ganglion of Aplysia has numerous cholinergic synaptic outputs on a large number of neurons in the ganglion. In addition to its chemical synaptic connections it has at least two electrical synaptic connections. The electrical junctions between L_{10} and the latter two small neurons is non-rectifying and it is possible to pass hyper- or depolarizing current pulses of various duration into any of these neurons and record attenuated hyperpolarizations or depolarizations in the others. With controlled spike activity in L_{10} chemical PSPs of regular amplitude can be evoked in the follower neurons. The imposition of hyperpolarization or depolarization on the electrically coupled neuron (L_{20}) during the activity of L_{10}, modifies the amplitude of the PSP produced by L_{10}. Depolarization of L_{20} increases and its hyperpolarization decreases PSP amplitude. With sufficient polarization of L_{20} the decrement or enhancement of PSPs produced by L_{10} can be up to 100% in some neurons. The PSPs in the LUQ of ganglion are most affected, the PSPs in the RLQ least affected while the PSPs in the LLQ are moderately affected. These effects are brought about due to certain anatomic relationships and biophysical properties of the terminal branches. When L_{20} is hyperpolarized, the transmitted polarization through the electrical synapse which is "presynaptic" to the chemical terminal of L_{10}, causes a hyperpolarization of the latter branch. Due to the anomalously rectifying properties of these neurons, resistance of the membrane decreases and the current produced by the oncoming action potential is shunted. Consequently transmitter release is diminished. Depolarizing currents have the opposite effects. These findings indicate an integrative role for electrical synaptic transmission whereby hyperpolarizations or depolarizations of the presynaptic neuron L_{20} can differentially affect transmitter release by the different terminals of L_{10}.

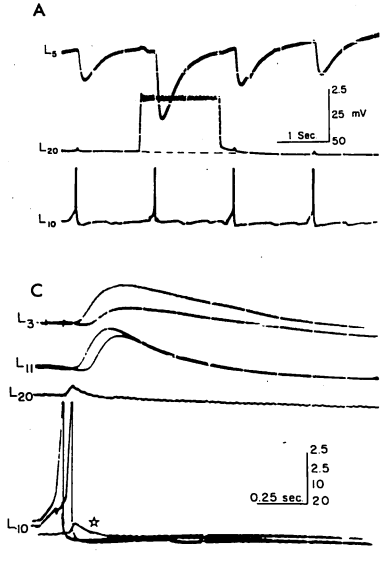

Fig. 1. Effects of depolarizing (A) and hyperpolarizing (C) pulses in the presynaptic neuron on the efficacy of chemical synaptic transmission by L_{10}. A. The activity of interneuron L_{10} produces chemical IPSPs in L_5 and small electrical EPSPs in L_{20}. A depolarizing pulse causing spike activity in L_{20} (full spikes not shown) causes a 100% increase in the amplitude of the next IPSP in L_5. C. IPSPs (inverted) in L_3 and L_{11} from L_{10} are differentially affected when a hyperpolarizing pulse is applied to L_{20}. Decrement in the amplitude of IPSP in L_3 is 60% while the IPSP decrement in L_{11}, is about 20% when L_{20} is hyperpolarized (star). As recorded, from the soma the action potential of L_{10} is unaffected as far as the amplitude is concerned, but with hyperpolarization there is a decrease in spike duration.

1442 AMPLITUDE AND DECAY RATE OF POST-TETANIC POTENTIATION CONTROLLED BY SEPARATE STIMULUS-HISTORY SENSITIVE SYSTEMS. Paul B.J. Woodson, Werner T. Schlapfer and Samuel H. Barondes. Dept. Psychiat., Sch. Med., UCSD, La Jolla, CA. 92093, and the V.A. Hospital, San Diego, CA. 92161.

At various synapses (J. Neurophysiol. 16:509, 1953; J. Physiol. (Lond.) 245:183, 1975; Schlapfer et al., Brain Res., in press, 1976), post-tetanic potentiation (PTP) decays with a single exponential time course, asymptotically approaching the size of an isolated post-synaptic potential (PSP). After longer or higher frequency trains of stimulation, PTP is larger (as a percentage of an isolated PSP) and decays slower. At a central synapse in Aplysia californica we showed (Schlapfer et al., op.cit.) that PTP amplitude increases as the number of stimuli in a train at 1/sec is raised; but for more than 300 pulses, the amplitude ceases to increase, although the decay rate continues to slow down. This finding suggests two stimulus history sensitive systems: one controlling the amplitude and another the decay rate. This hypothesis was supported by showing the decay rate could be manipulated independently of the amplitude by lowering the temperature of the preparation (Nature 258:623, 1975) or by addition of 0.8 M ethanol to the perfusate (Nature 260:797, 1976). At low temperature PTP decays slower, with ethanol it decays faster; but the amplitude is unaffected in both cases. In this abstract we show the converse contrast, i.e., the amplitude of the PTP may be manipulated independently of the decay rate.

We studied the monosynaptic, unitary, cholinergic, Cl^- dependent IPSP elicited in cell L5 of the abdominal ganglion of A. californica upon stimulation of cell L10 (J. Neurophysiol. 34:76, 1971). L10 was hyperpolarized 20 mV below its resting potential to prevent spontaneous firing. A train of 150 pulses at 1/sec gave a PTP with a single exponential time course of decay with a rate constant of 0.01/sec and an amplitude of 250% of an isolated PSP (av., n=9). A train of 600 pulses decreased the PTP decay rate to 0.003/sec and increased the amplitude to 350% (av., n=4).

The first method used to change PTP amplitude was elevation of Ca^{++} in the perfusate. In 5x Ca^{++} the PTP amplitude was only 140% of an isolated PSP after 150 pulses at 1/sec; the isolated PSPs were increased much more than the PTP PSPs by high Ca^{++}. In contrast, the decay rate was unchanged in high Ca^{++} (av., n=4). A second method of varying the PTP amplitude was to change transmitter release by hyperpolarizing the presynaptic cell. In confirmation of Shimahara and Tauc (J. Physiol. (Lond.) 247:299, 1975) hyperpolarization of L10 led to a decrease in the PSP in L5. This effect is not mediated by an electrotonic action upon the post-synaptic cell, since a given change in the polarization of the presynaptic neuron produces the same magnitude and direction of changes in the PSP amplitude, regardless of whether the PSP in L5 is an IPSP or an EPSP, as controlled by the polarization level of L5. We conclude that presynaptic polarization reduces transmitter release; indeed, addition of Ca^{++} to the purfusate antagonized the effects of hyperpolarization of L10. When the size of an isolated PSP was reduced to 20% of control by hyperpolarization of L10 -70mV below its resting potential, the amplitude of the PTP generated by a train of 150 pulses at 1/sec was increased to 600% of an isolated PSP. This was because the presynaptic hyperpolarization decreased PTP PSPs much less than isolated PSPs. Again, the PTP decay rate was unaffected (av., n=9).

We conclude: 1)PTP amplitude and decay rate are under the control of separate stimulus history sensitive systems; 2)PTP amplitude correlates with the amount of transmitter released by an isolated spike when the amount is varied by changes in Ca^{++} or presynaptic polarization level; and 3)PTP decay rate is independent of the level of transmitter release when the latter is varied by these manipulations.

1443 POSTSYNAPTIC CONDUCTANCE CHANGES DURING PRESYNAPTIC INHIBITION. P. L. Carlen*, Y. Yaari* and R. Werman* (SPON: R. D. G. Blair). Department of Neurobiology, Hebrew University, Jersalem, Israel.

The effects on postsynaptic motoneuron membrane conductance of minimal presynaptic conditioning stimuli which decreased the height of an intracellularly measured Ia EPSP in adult cat spinal cord were measured. The usual conditioning stimuli were 4 shocks, 4 msec apart, to the posterior biceps semitendinosus nerve at less than 1.3 X threshold. The tested Ia EPSP was usually elicited from lateral gastrocnemius nerve approximately 100 msec after the first conditioning stimulus. Only cells showing no evidence of long-lasting IPSP's on superimposed single sweeps were used. Postsynaptic conductance changes were measured by analyzing the decay of a short (.3 msec) intracellular constant current (1-10 nA) pulse (Jack & Redman, J. Physiol. 215: 321, 1971) which was generated at the same time interval after the first conditioning stimulus as was used for a previously inhibited Ia EPSP. Up to 120 sweeps at 1 Hz were averaged and the decay of a conditioned short pulse was compared to the decay of an unconditioned short pulse. In 22/23 cases, where the Ia EPSP was inhibited by conditioning stimuli, the conditioned short pulse decayed faster than an unconditioned short pulse. This faster decay was evident even when the heights of conditioned and unconditioned saturating current pulses were identical. With averaging of the baseline, a long-lasting IPSP was evident in 10/15 cells. Analysis of the membrane time constant, τ_0, and the first equalizing time constant, τ_1, from the short pulse decay, according to the Rall model of the motoneuron, suggested in many cases that the increased conductance measured after the conditioning stimuli occurred mainly in the more distal dendrites.

1444 PURIFICATION OF SYNAPTIC VESICLES. Steven S. Carlson*, John A. Wagner* and Regis B. Kelly. Dept of Biochemistry & Biophysics, University of California, San Francisco 94143.

Understanding the mechanism of neurosecretion requires isolation and study of the sub-cellular components of the nerve terminal. We have developed a procedure for preparing synaptic vesicles from the electric organ of the ray, Narcine brasiliensis, which yields vesicles homogeneous in size. To minimize discharge of vesicles during preparation, we perfused the anesthetized ray with an isoosmotic solution containing 10 mM EGTA before removing the electric organ. The electric tissue was homogenized in a medium containing 0.8 M sucrose and debris was removed by centrifugation at 16,000g for 30 min. The vesicles were isolated from the supernatant by sucrose density flotation centrifugation. This procedure removes most of the contaminating soluble proteins. The vesicle fraction is then purified further by molecular sieve chromatography on a Controlled Pore Glass-10 (3000Å pore size) column. By phospholipid phosphorus determinations only two membrane components are present in the elution profile, one totally included and one present in the void volume. All of the ATP and acetylcholine are associated with the included component and are coincident with the peak of phospholipid. This indicates that the synaptic vesicle preparation is homogeneous at least with respect to size. The phospholipid/protein ratio (3.9 moles phospholipid/mg protein) was consistent with the density (1.05 g/ml) of these acetylcholine vesicles, but much higher than that published for Torpedo marmorata vesicles which have the same buoyant density. These vesicles are more pure by biochemical criteria than earlier preparations, and they seem to have remarkably little protein relative to phospholipid, suggesting that earlier preparations of synaptic vesicles might have been contaminated with protein. Supported by NIH grant #NS09878. SC(NS01365) & JW(NS05092) are NIH postdoctoral fellows

1445 POSSIBLE ROLE OF PHOSPHOPROTEINS IN MEDIATING CALCIUM-DEPENDENT NEUROTRANSMITTER RELEASE. Robert J. DeLorenzo. Dept. Neurology, Yale Univ. Sch. Med., New Haven, Ct. 06510.

Posttetanic potentiation (PTP) appears to be the result of an increase in neurotransmitter release secondary to an intracellular accumulation of calcium ions in the nerve terminal during repetitive stimulation (Weinreich,J. Physiol. 212:431,1971). The calcium dependent increase in neurotransmitter release during PTP can be inhibited by the anticonvulsant diphenylhydantoin (DPH). DPH has also been shown to inhibit the calcium dependent release of norepinephrine from brain slices (Pincus and Lee, Arch. Neurol. 29:239,1973). Studies in this laboratory have demonstrated that DPH and calcium act antagonistically on the phosphorylation of specific brain proteins, and suggested that the opposing effects of DPH and calcium on neurotransmitter release during PTP and from brain slices might be mediated through their antagonistic actions on the phosphorylation of specific brain proteins. The effects of calcium and DPH on the endogenous phosphorylation of specific proteins in synaptosomal preparations were studied using the technique of SDS-acrylamide gel electrophoresis. The phosphorylation of two synaptosomal fraction proteins, designated proteins DPH-L and DPH-M (m.w., 62,000 & 51,000,respectively) were completely dependent upon the presence of calcium ions. The calcium induced stimulation of incorporation of [^{32}P] phosphate from [γ-^{32}P] ATP into these proteins was inhibited by DPH by more than 80%, at concentrations of DPH that could reverse PTP and inhibit norepinephrine release from brain slices. The results are compatible with the hypothesis that the antagonistic effects of calcium and DPH on the release of neurotransmitter during PTP and from brain slices might be mediated by the antagonistic action of these agents on the phosphorylation of specific synaptosomal proteins.

1446 SYNAPTIC TRANSMISSION AND THE EFFECT OF NOREPINEPHRINE ON THE ISOLATED SUPERIOR CERVICAL GANGLION OF THE GUINEA PIG. N. Dun* and A. G. Karczmar (SPON: R. S. Schmidt). Dept. Pharmacol. Loyola Univ. Med. Ctr., Maywood, IL 60153.

Single supramaximal preganglionic stimulation applied to the nicotinized sympathetic ganglion cell of the guinea pig elicited no detectable postsynaptic potential; whereas, a tetanic stimulation (10-30 Hz, 1-5 sec) consistently evoked a slow depolarizing potential resembling the slow excitatory postsynaptic potential. No hyperpolarizing potential was detected prior to the generation of the depolarizing potential. In non-nicotinized preparations, norepinephrine (NE) (1-10 μM) consistently suppressed the amplitude of fast excitatory postsynaptic potential (fast e.p.s.p.) elicited by submaximal preganglionic stimulation and eventually blocked the synaptic response; the effect of NE was readily reversible. The resting membrane potential, total membrane resistance, and the amplitude and time course of the ganglion cell action potential were all not significantly affected by NE. Pretreating the ganglion with phenoxybenzamine (10 μM) completely prevented, whereas propranolol (30 μM) failed to antagonize, the ganglionic depressant action of NE. The amplitude and time course of the iontophoretically induced ACh-potential were not appreciably altered by NE in concentrations which markedly attenuated the response of fast e.p.s.p. Altogether, the results suggest that neither the slow inhibitory postsynaptic potential nor NE-hyperpolarization are involved in the blockade of synaptic transmission of the sympathetic ganglion cells of the guinea pig and that NE inhibits transmission by mainly acting at a presynaptic alpha-adrenergic site to reduce the output of acetylcholine from the nerve terminals.

(Supported by NSO6455).

1447 EVIDENCE THAT A PROSTAGLANDIN (PG) SYSTEM CONTROLS CHOLINERGIC TRANSMISSION IN THE GUINEA PIG ILEUM. S. Ehrenpreis, J. Greenberg* and J.E. Comaty*. N.Y.State Research Institute for Neurochemistry and Drug Addiction, Ward's Island, New York 10035

We have recently proposed that opiates inhibit transmission in guinea pig ileum by blocking the action of PG which functions as a modulator for acetylcholine release (Ehrenpreis et al Nature New Biol. 245: 280, 1973). We have now obtained more direct evidence for the essentiality of the PG system in transmission by examining effects of PG synthetase inhibitors and PG receptor blockers. All of these compounds block electrically-induced contractions of the longitudinal muscle-myenteric plexus preparation. ED_{50}'s for the synthetase inhibitors are as follows: diclofenac sodium $6x10^{-7}$M, indomethacin $1.3x10^{-6}$M, ETA $4.0x10^{-6}$M, phenylbutazone $7x10^{-5}$, and aspirin $7x10^{-4}$. The order of potency correlates almost perfectly with the Ki of these compounds for the synthetase (Ku and Wasvary, Biochim. Biophys. Acta 384:360, 1975). ED_{50}'s for the PG receptor blockers SC19220 and 7-0xa-13prostynoic acid are $2.3x10^{-5}$ and $2x6x10^{-5}$M respectively. Inhibitory effect of all of these compounds is reversed selectively by PGE_1 and E_2 at 2-4ng/ml. SC19220 inhibits binding of PGE_2 to homogenates of ileum with a Ki of $2x10^{-5}$M. Block of transmission occurs with little effect on response to exogenous ACh showing pre-synaptic site of action. Physostigmine is equally potent in reversing block by indomethacin and morphine, showing that both drugs have a similar mechanism of action, namely, decreased output of ACh. These results provide strong support for the concept that a PG receptor and PG synthetase are present in myenteric plexus of guinea pig ileum and are essential for transmission in this tissue. (Supported in part by NIDA grant 00496.)

1448 LASER LIGHT SCATTERING FROM THE PERICARDIAL ORGAN OF THE CRAB, CARCINUS - EFFECT OF POTASSIUM PERFUSION. David Englert* and Charles Edwards. Dept. Biol. Sci., SUNYA, Albany, N.Y. 12222.

Spectral analysis of intensity fluctuations of scattered laser light can give information on the dynamics of submicroscopic particles, e.g.- macromolecules or cellular organelles. Random motion of such particles causes Doppler broadening of the light, resulting in light beating and a monotonically decreasing fluctuation spectrum. It has been reported that perfusion of neurosecretory tissues with elevated potassium concentrations causes a reversible increase in the amplitude of the light beating spectrum, suggesting a coupling between nerve ending depolarization and increased organelle motion (D.B. Sattelle and R.W. Piddington, J. Exp. Biol. 62:753).

We have investigated this phenomenon, using the pericardial organ of the crab, Carcinus. Experiments were done on a specially modified microscope to be described.

Potassium perfusion causes a broadening and an increase in amplitude of the intensity fluctuation spectrum, which is graded with potassium concentration and at least partially reversible. Spectra show q^2 dependence, characteristic of translational motion. Calcium ions are not required in the bath. The effect is much smaller if the chloride ions are replaced with relatively impermeant methane sulfonate ions, and can also be produced by diluting the normal bath solution.

The results indicate that the neurosecretory vesicles are probably bound in a gel matrix in the nerve endings and that KCl perfusion causes swelling and disruption of the axoplasm, allowing the vesicles to undergo diffusion.

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Lioresal depresses synaptic transmission in the cuneate nucleus. S. Fox, K. Krnjević & M.E. Morris (SPON: R. B. Malmo), Anaesthesia Research, McGill University, Montreal, Canada.

Intravenous injections of Lioresal (β(4-chlorophenyl)γ-aminobutyric acid Ciba-Geigy Ltd.) in decerebrate cats ($\geqslant$0.5 mg/kg) cause a gradual and prolonged reduction in transmission efficiency, while afferent terminals consistently become less excitable; but there is little depression in post-synaptic excitability (as indicated by the α wave of the lemniscal potential) or of the inhibition by a conditioning volley (evoked by stimulating a forelimb nerve). Although doses as small as 1 mg/kg can reduce transmission by almost half, even 11 mg/kg may not produce a complete block, presumably owing to the normally high probability of transmission through many cuneate synapses. The action of Lioresal is very remarkable for several reasons. No other agent in such low systemic doses blocks primary afferent synapses so effectively (probable tissue concentration 10^{-6}-10^{-5} M). Although it is even more potent than GABA in raising the input conductance when applied directly to spinal motoneurons (Puil *et al.*, 1975, *Fed. Proc. 35*, 307), such an effect probably requires a much higher concentration. In agreement with other authors (Pierau & Zimmermann, 1973, *Brain Res. 54*, 375; Davidoff & Sears, 1974, *Neurology 24*, 957), we conclude that Lioresal probably acts by depressing transmitter release. The fall in terminal excitability runs counter to the inverse relationship between terminal excitability and efficiency of transmission commonly observed in the cuneate nucleus (Krnjević & Morris, 1976, *J. Physiol.*, in press), and provides further evidence (*cf.* Davidoff & Sears, 1974) against the suggestion that Lioresal acts by depolarizing presynaptic fibres (Pierau & Zimmermann, 1973).

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1450 RETRIEVAL OF VESICLE MEMBRANE AFTER "DEPOLARIZATION" IN SYNAPTOSOMES. Robert C. Fried* and Mordecai P. Blaustein. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

Pinched-off presynaptic terminals ("synaptosomes"), prepared from rat brain homogenates, were incubated in physiological salt solutions containing a normal (5mM) or elevated (50mM) K concentration. Some of the 5mM K solutions contained 75μm veratridine and/or 100nM tetrodotoxin (TTX). An extracellular marker, either horseradish peroxidase or colloidal thorium dioxide, was sometimes present in the incubation fluids. Following incubation, the synaptosomes were fixed with glutaraldehyde and prepared for electron microscopy. Synaptosomes incubated in the standard (5mM K) saline generally contained numerous uniform-diameter (~500Å) synaptic vesicles and normal-looking mitochondria; occasional large vesicles, coated vesicles and vacuoles were observed. Extracellular markers were excluded from most of the synaptosomes - implying that the surface membranes are resealed. Following exposure to media containing 50mM K or 5mM K + 75μM veratridine (a depolarizing agent) the number of synaptic vesicles per unit cross-sectional area decreased; the number of large diameter (900Å) vesicles and coated vesicles, and the percentage of terminals containing large vacuoles increased. Some large vesicles could be seen in various stages of detachment from the plasmalemma. When the incubation solutions contained an extracellular marker along with a depolarizing agent, the marker was seen in many large vesicles, coated vesicles, and vacuoles. The morphological changes observed in the "depolarized" synaptosomes were largely prevented when Ca was omitted from the medium, or when TTX was added prior to veratridine. These results indicate that synaptosomes release transmitter by an exocytotic process and that the membrane is subsequently retrieved from the surface by endocytosis.

1451 DECREMENT AND DESENSITIZATION OF A SELF-INHIBITORY SYNAPTIC POTENTIAL (SISP) IN APLYSIA BUCCAL GANGLIA. Daniel Gardner. Dept. of Physiology, Cornell Univ. Medical College, New York, N.Y. 10021.

The buccal ganglia of Aplysia contain four identified cholinergic interneurons, each of which monosynaptically inhibits itself, producing a self-inhibitory synaptic potential (SISP) after each action potential. Voltage clamps show a curare (dTC)- and Mg-sensitive voltage-independent conductance change which peaks, then decays exponentially. The reversal potential is ≅-65mV. The conductance change persists without failure in threshold-raising high Ca & Mg solutions, suggesting monosynapticity.

I now report that both SISP and conductance change decrement rapidly with repetitive stimulation or short inter-spike interval (ISI). Much of this decrement appears due to desensitization of the cholinergic postsynaptic receptor mediating the SISP. In particular: 1)Peak conductance decreases as log(ISI); the decrement is not accompanied by a latency change. 2)Similar currents seen in a variety of preparations have been ascribed to a Ca-activated K conductance. A further test for distinguishing whether the SISP is due to a synapse or to a Ca-activated current is possible because the receptor assumed responsible for the SISP readily desensitizes to acetylcholine (ACh). Accordingly, ganglia were bathed in 1mM ACh in order to desensitize the cholinergic SISP receptor. Subtracting currents obtained in ACh from those obtained in sea water revealed an excess-ACh sensitive current predicted by assuming a cholinergic basis to the SISP but not consistent with a Ca-activated K conductance. Excess-ACh sensitive currents resembled Mg- and dTC-sensitive currents in shape, amplitude, decay time constant, and reversal potential. 3)The rapid decrement of the SISP ensures that functional self-inhibition is transient: most effective at the start of excitatory input or at certain rates of firing.

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1452 SYNAPTIC CONNECTIVITY IN TUBERAL HYPOTHALAMIC TISSUE CULTURES: PHARMACOLOGICAL EVIDENCE. H. M. Geller, Dept. of Pharmacology, Rutgers Medical School, Piscataway, NJ 08854.

Several different patterns of spontaneous activity have been recorded from cultured tuberal hypothalamic neurons. These patterns include slow, irregular discharge and both slow phasic (periodicity of 1-2 min) and fast phasic (bursty, periodicity 5-10 sec). After raising the Mg^{2+}/Ca^{2+} ratio in the perfusion fluid, the slow, phasic pattern became irregular, suggesting that this pattern is generated by synaptic activity. In order to directly test for the influence of synaptic connections, we have used focal electrical stimulation in combination with extracellular unit recording, and have computed post stimulus time histograms of the resulting activity patterns. Both antidromic and orthodromic excitations were observed, along with inhibitions. These effects were graded with stimulus intensity. Specific blocking agents were used to identify the transmitters responsible for the observed inhibitions; either strychnine and picrotoxin in 10^{-5} M concentration, but not both, were able to reduce the duration of electrically induced inhibitions of activity. These results suggest that there exist excitatory and inhibitory synapses in these cultures, and that both glycine and GABA function as inhibitory transmitters in the tuberal region of the hypothalamus. (Supported by USPHS grant no. NS 11295)

1453 INHIBITION BY BOTULINUM TOXIN OF POTASSIUM-INDUCED RELEASE OF ACETYLCHOLINE FROM BRAIN SLICES. Cameron B. Gundersen, Jr.* and Bruce D. Howard* (SPON: C. D. Clemente). UCLA Medical School, Los Angeles, CA 90024.

Slices of mouse cerebrum were incubated at 37° in the presence of 8 X 10^3 mouse MLD_{50} of type A botulinum toxin per ml. Control slices were treated the same way with heat-inactivated toxin. After two hours the slices were washed by centrifugation and incubated for 20 minutes in a buffer containing 0.1 mM physostigmine and either low K^+ (5 mM) or high K^+ (35 mM). After centrifugation acetylcholine levels in the media and tissues were determined by gas liquid chromatography/mass spectrometry by the method of Freeman et al. (J. Neurochem. 24; 729, 1975). Incubation of control slices in high K^+ buffer produced at least a two fold increase in acetylcholine efflux compared to incubation in low K^+ buffer. Thus, after prolonged incubation K^+ remained capable of eliciting an increased acetylcholine efflux. Treatment with botulinum toxin reduced the K^+-evoked efflux of acetylcholine by 20-60%. This reduction could not be attributed to a decrease in the total level of acetylcholine in the slices treated with botulinum toxin. These studies suggest the utility of botulinum toxin as a probe of cholinergic mechanisms in the central nervous system and particularly of intracellular acetylcholine turnover and distribution.

1454 FACILITATION AND DEPRESSION OF EXCITATORY SYNAPTIC INPUT TO CEREBELLAR PURKINJE CELLS. J. T. Hackett and S. L. Cochran, Dept. Physiol., Univ. of Virginia, Charlottesville, VA 22901.

Modification of synaptic transmission is commonly achieved at chemical synapses following repetitive stimulation. The two excitatory synaptic inputs to a Purkinje cell (PC), the parallel fibers (PF's) and a single climbing fiber (CF), react differently to repetitive stimulation; the former is facilitated and the latter is depressed. To investigate the site and mechanism of action of repetitive stimulation, experiments were done on frog cerebella in vitro (Hackett, 1972). Repetitive stimulation of PF's increased extracellularly recorded PF volley, which is attributed to an increase in number of activated PF's. Unitary EPSP's evoked by 1 Hz CF stimulation had a constant amplitude within each PC and a very low coefficient of variation (CV), indicating a large quantal content. Higher stimulation frequencies depressed CF-EPSP with no change in CV. The failure to detect quantal release is probably related to non-Poissonian processes and to non-linear summation of EPSP amplitudes. Decreasing $[Ca^{2+}]_o$ 4 fold reversibly decreased CF-EPSP amplitude. After 5 minutes, fluctuations in CF-EPSP amplitude and occasional failures in CF-PC transmission occurred. Reduced CF-PC transmission could be a Poisson process. Quantal content calculations based on this assumption indicate that Mn^{2+} and low Ca^{2+} greatly reduce the number of quanta released. With transmission reduced, repetitive stimulation resulted in facilitation of CF-EPSP amplitudes and increased quantal content. Miniature CF-EPSP's can be identified, but they are rarely detected. Thus, assessments of quantal size variation are difficult. In agreement with results at other synapses where quantization is evident, CF-PC synaptic facilitation is most likely due solely to increased quantal content. Uncertainties will be settled by more reliable estimates of quantal size and identification of CF neurotransmitter substance.

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1455 REEVALUATION OF THE STRUCTURAL EFFECTS OF LANTHANUM ON THE FROG NEUROMUSCULAR JUNCTION WITH A NEW FLASH-FREEZING TECHNIQUE. John E. Heuser* (SPON: E. Mayeri) Dept of Physiology, Univ of California, San Francisco

Nerve terminals in frog cutaneous pectoris muscles which were processed by a new method of freeze-substitution which will be described, after they were treated with 2mM La^{+++} for specific periods of time, showed the following structural changes: (1) Expansions and dislocations of the presynaptic plasmalemma consequent to massive exocytosis of synaptic vesicles could be seen directly, as well as measured, thus confirming Clark et al's views (J.C.B.52:1). (2) Proliferation of cisternae and other internal membrane forms representing vesicle membrane retrieved from the plasmalemma could be seen even more clearly than in conventionally-fixed material, confirming the existance of vesicle membrane recycling at this synapse (Heuser & Reese, J.C.B.57:315). (3) Structural details of the involved membranes and their adjacent cytoplasm and filaments confirm the involvement of 'coated vesicles' in membrane recycling, and further support the idea that the 'clathrin' coats of these vesicles (Pearse, P.N.A.S.73:1255) play a role in the selective retrieval of specifically synaptic vesicle membrane as they pinch off from the presynaptic plasma membrane (Heuser & Reese, Anat Rec.181:374).

1456 THE EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$) ON NERVE CONDUCTION. Kenneth I. Kohn* and Mary Ann Marrazzi, Department of Pharmacology, Wayne State University School of Medicine, Detroit, Michigan 48201.

Prostaglandins (PGs) have been shown to inhibit synaptic transmission in various brain regions including cerebral cortex (Marrazzi et al.), cerebellar cortex (Siggins et al.), and cuneate nucleus (Coceani et al.). However, that PGs affect neuronal conduction remains a possibility. Indeed, the known restraint of calcium (Ca^{++}) efflux in smooth muscle might be an important aspect of PG modulation of excitable tissues. The restraint and subsequent motions of ions by electrical forces, ion pumps or carriers describes the functioning membrane which triggers excitation-be it muscle contraction, secretion, or neuronal conduction or transmission. This membrane is most clearly approachable as a separate ionic system in neuronal conduction. Conduction block by local anesthetics has been attributed to effects on Ca^{++} mediated stabilization. Hence, the effects of $PGF_{2\alpha}$ on nerve conduction were studied.

Modulating influences are most likely to have major consequences in submaximal situations. Therefore, conduction elicited by submaximal stimulation was recorded in isolated frog sciatic nerve in a moist chamber arranged so that a segment could be exposed to $PGF_{2\alpha}$ (10^{-7} to 10^{-5} M) and/or a known blocker, procaine (5 mM) in normal or 1/3 normal Ca^{++}. It was demonstrated that $PGF_{2\alpha}$ 1) did not alter the stimulus-response curve or the amplitude of the action potential in either normal or 1/3 normal Ca^{++}; 2) did not significantly alter procaine blocking in normal or 1/3 normal Ca^{++}. Unlike smooth muscle contraction, synaptic transmission or NE release, neuronal conduction was not influenced by $PGF_{2\alpha}$. Thus, localization of PG modulation of neuronal consequences needs to focus exclusively on synaptic elements and on excitation-contraction or excitation-transmission coupling. Supp. NIH GRS RR05384.

1457 ANALYSIS OF SYNAPTIC FACILITATION AND ANTIFACILITATION USING RANDOM STIMULATION OF A PRESYNAPTIC AXON. Howard I. Krausz, W. Otto Friesen. Dept. Neurosciences, Sch. Med., UCSD, San Diego, CA. 92093.

A new method of nonlinear system identification, analagous to the Wiener method, was developed and applied to the study of a unitary facilitating synapse in the cardiac ganglion of the lobster Panulirus interruptus. The amplitudes of EPSP's evoked by arbitrary patterns of presynaptic impulse activity may be predicted from a series of functionals similar to a Volterra series, involving the system input and a set of kernels. When the input impulse train forms a random Poisson process, the functionals in the series are mutually orthogonal and their kernels may be estimated by input-output crosscorrelations. A simplified system identification procedure was developed for cases where all impulse responses rise sufficiently rapidly and then follow a similar time course. The procedure was applied to both a living synapse and to a mathematical model of the synapse constructed by Friesen to account for data from earlier experiments which used conditioning volleys followed by test impulses. As expected, the orthogonal series characterization of the model improved in accuracy each time another functional was added to the series. Experimentally measured kernels agreed with model kernels except for minor differences. It is concluded that a third order characterization of the synapse will capture many of its nonlinear features, but will not be quite as accurate as the Friesen model. Nevertheless, a Poisson impulse train analysis provides a comprehensive and objective comparison between experiment and model and yields a convenient and useful summary of a system's input-output behavior. (Supported by grants from the Sloan Foundation and NSF GJ-41809).

1458 CALCIUM-DEPENDENT PROTEIN PHOSPHORYLATION IN RAT BRAIN SYNAPTOSOMES. Bruce K. Krueger, Javier Forn*, and Paul Greengard. Dept. Pharmacology, Yale University School of Medicine, New Haven, Ct 06510.

Agents known to increase Ca^{2+} transport across nerve membranes were tested for their effects on endogenous protein phosphorylation in synaptosomes prepared from rat cerebral cortex. For this purpose, synaptosomes were preincubated in HEPES-buffered Krebs-Ringer's medium containing tracer amounts of carrier-free $^{32}P_i$ for 30 min prior to incubation with the various test agents. Veratridine, 60 mM K^+, and the calcium ionophore A23187 each stimulated the incorporation of ^{32}P into two specific proteins (apparent molecular weights 80,000 and 84,000) as determined by SDS-polyacrylamide gel electrophoresis [method of Studier, J. Mol. Biol. (1973) 70, 237] and autoradiography. All three agents failed to stimulate protein phosphorylation in calcium-free medium containing EGTA. Veratridine, K^+, or A23187 also stimulated $^{45}Ca^{2+}$ accumulation by synaptosomes. Tetrodotoxin blocked the stimulation of both protein phosphorylation and $^{45}Ca^{2+}$ accumulation by veratridine but not by K^+ or A23187. Cyclic AMP, 8-bromo-cyclic AMP, cyclic GMP, 8-bromo-cyclic GMP, norepinephrine, dopamine, acetylcholine, and 3-isobutyl-1-methyl xanthine were without effect on protein phosphorylation. Little or no ^{32}P was incorporated into protein when synaptosomes were subjected to hypo-osmotic shock prior to incubation with $^{32}P_i$. The data suggest that conditions which cause an accumulation of calcium by synaptosomes lead to the calcium-dependent phosphorylation of specific endogenous proteins. The results raise the possibility that certain calcium-dependent nerve terminal functions, such as neurotransmitter release or synthesis, may be regulated by the phosphorylation of these proteins.

1459 QUANTAL TRANSMISSION AT THE SQUID GIANT SYNAPSE.
Diana W. Mann and Ronald W. Joyner*. Institute of Marine Biomedical Research, UNC-W, Wilmington, N. C. 28401, and Dept. of Physiology, Duke University School of Medicine, Durham, N. C. 27710.

The Squid Giant Synapse offers the opportunity to study the relationship between presynaptic activity and transmitter release. However, the small size of the miniature synaptic potentials (MAPs) in adult squid (<5 μv)has prevented any quantal analysis at this synapse. In small squid of 2-6cm mantle length and giant axon diameters of 75-140μm, MSP amplitudes are an order of magnitude larger. This fact, combined with digital signal processing, has enabled us to record MSPs and investigate the quantal nature of synaptic transmission.

Standard recording techniques were used, data stored on tape and computer analyzed. Digital techniques were used to discriminate MSPs from noise of two types: recording noise of 10-30μv and background "biological noise" of 20-35μv. The latter was also analyzed as to content and source. MSP amplitudes for the distal synapse were 60-120 μv in different preparations.

The MSPs were studied as to amplitude distribution and frequency as functions of time, temperature, high frequency (10-50 Hz) conditioning stimulation, $[Ca]$ and $[La]$. Quantal contents of the EPSP were reduced below firing threshold by the addition of La^{+3} to the artificial seawater (ASW) perfusing the ganglion. The unitary EPSP amplitudes recorded in La-ASW correlated with the spontaneous MSP amplitudes. Amplitude fluctuations of the EPSP under these conditions confirmed the quantal nature of transmission at this synapse.

1460 EFFECTS OF ANAESTHESIA ON SYNAPTIC TRANSMISSION IN THE CUNEATE NUCLEUS.
Mary E. Morris. Department of Anaesthesia Research, McGill University, Montreal, Quebec, Canada.

Although anaesthesia is generally believed to depress transmission at synapses of the central nervous system, the action on the primary afferent relay of at least one highly secure pathway - the dorsal column-lemniscal system - is, in striking contrast, a facilitatory one. In experiments in decerebrate cats, which were paralyzed and received constant-volume ventilation, halothane (0.5-2.0%) was briefly administered (3-10 minutes), or methohexital (1-30 mg), pentobarbital (10-100 mg), althesin (3.6-24 mg), or ketamine hydrochloride (25-75 mg) were given intravenously. The cuneate synaptic output for a given input - as estimated from antidromic and transynaptic responses to intra-nuclear stimulation of the afferent terminals (*Krnjević & Morris, J. PHYSIOL., in press, 1976*) - was most commonly enhanced by anaesthesia, while at the same time terminal excitability decreased. Cuneate synaptic responses to stimulation of forelimb nerves were also potentiated. These effects occurred within 1-2 minutes and were reversible. Occasional diminution of synaptic efficacy, usually transient and associated with increased terminal excitability, was consistently related to concomitant hypotension; these opposing changes were most frequently observed shortly after the start of anaesthesia, as arterial pressure was falling. The depression of terminal excitability by anaesthesia suggests a hyperpolarizing action on the cuneate afferent terminals - perhaps due to increased K^+ conductance or electrogenic Na-K pumping. Although this might produce invasion block in presynaptic regions, the evidence of facilitation of the synaptic transmission evoked by afferent volleys points rather to augmentation of transmitter release as being the principal effect.
(*Supported by the Medical Research Council of Canada*).

1461 POTASSIUM-INDUCED RELEASE OF GABA FROM SYNAPTOSOMES IS AN ENERGY-DEPENDENT PROCESS. Diana C. Nelson-Krause* and Bruce D. Howard* (SPON: M. Philippart). UCLA Medical School, Los Angeles, CA 90024.

We have examined the energy requirements of depolarization-induced release of GABA from synaptosomes prepared from rat cerebral cortex. The synaptosomes, which had been pre-loaded with [^{14}C]GABA, were incubated at 30° for 15 min with various inhibitors of energy metabolism and then exposed to 30 mM K^+ for 1 min. This procedure caused a Ca^{2+}-dependent efflux of GABA from control synaptosomes not treated with an inhibitor. Depletion of energy stores, per se, caused a GABA leakage that could be prevented by incubation in a Na^+-free buffer. While Na^+ was required for loading the synaptosomes with [^{14}C]GABA, it was not necessary for the K^+-induced release of GABA. Therefore, after loading, all incubations were in a Na^+-free buffer. Treatment with 3 mM sodium azide or 0.02 μM S-13 (5-chloro-3-t-butyl-2'-chloro-4'-nitrosalicylanilide), a potent uncoupler of oxidative phosphorylation, inhibited K^+-stimulated GABA release by 70%. Release could also be inhibited by several trialkyltin compounds, which are known to inhibit mitochondrial ATPase. The most potent was tributyltin, which at 0.1 μM completely inhibited GABA release. At this concentration tributyltin had no effect on the high affinity uptake of GABA indicating that it was not inhibiting K^+-induced GABA release by some non-specific destruction of membranes or by inhibiting mitochondrial ATPase alone. This latter conclusion is supported by the finding that another mitochondrial ATPase inhibitor, oligomycin, at 4 μg/ml had no effect on the K^+-induced release of GABA. Therefore, tributyltin may act by affecting some other ATPase involved in transmitter rlease. We have found tributyltin inhibited a Ca^{2+}/Mg^{2+}-ATPase associated with a fraction enriched in synaptic vesicles from rat brain.

1462 THE PHYSIOLOGICAL MODE OF ACTION OF A PRESYNAPTIC NEUROTOXIN ISOLATED FROM BLACK WIDOW SPIDER VENOM. R. L. Ornberg* and T. Smyth, Jr.* (SPON: R. Ravizza). The Pennsylvania State University, University Park, PA 16802

Disc gel electrophoresis has been used to isolate a protein from black widow spider venom which causes a large transient increase in the miniature endplate potential (mepp) frequency at cockroach neuromuscular synapses. This slowly migrating protein toxin (Rf 0.40, MW 125,000, 8-10S) has no sugar residues, no lipid residues, and no detectable protease, esterase, or glucosidase enzyme activity. The effect of this toxin on resting transmitter release has been recorded in a variety of saline solutions and is dependent on external sodium. In low Na^+ saline (100 mM) the toxin-induced increase in mepp frequency is delayed. Ouabain accelerates this increase. Intracellular calcium is probably important also for toxin-induced transmitter release because it can be regulated by compounds which are capable of chelating calcium ions. This evidence suggests the following sequential action for this toxin; a primary action allows sodium ion influx. This causes the release of calcium from intracellular stores which in turn accelerates transmitter release.

1463 NET UPTAKE OF γ-AMINOBUTYRIC ACID BY A HIGH AFFINITY SYNAPTOSOMAL TRANSPORT SYSTEM. Robert Roskoski, Jr. and Leslie D. Ryan*, Dept. of Biochemistry, The University of Iowa, Iowa City, Iowa 52242 U.S.A.

Reuptake of γ-aminobutyric acid (GABA) by a high affinity transport system by nerve endings in the central nervous system is postulated to terminate the action of this alleged neurotransmitter. Since net concentrative uptake is required to inactivate by this mechanism, this idea has been challenged since the demonstration by Levi and Raiteri (Nature 250, 735 (1974)) of exchange between low concentrations of exogenous GABA and synaptosomal GABA. We also find appreciable GABA exchange under similar experimental conditions. To extend these studies, GABA deficient synaptosomes were collected by centrifugation after KCl depolarization in the presence of $CaCl_2$. The procedure decreased endogenous GABA from 9.5 to 7 nmol GABA/mg protein. These synaptosomes were incubated with 10 μM ^{14}C-GABA in Ringers solution containing 1-4 mM KCl at 25° (4 min). At this concentration, most of the GABA uptake is mediated by the high affinity transport system. After centrifugation, total (fluormetric) and labeled GABA were measured in the supernatant. With the depleted synaptosomes, apparent (radiolabeled) GABA uptake closely paralleled the total uptake. Furthermore, the apparent and net uptake are identical at pH 6.0-8.5, protein concentrations from 0.4-3.5 mg/ml and temperatures from 5-37°. Exchange which predominates with higher endogenous GABA (9.5 nmol/mg protein) may reflect the finite capacity of the storage system. Our experiments demonstrate net retention of ^{14}C-GABA by GABA-deficient synaptosomes; this finding is consistent with the notion that the high affinity transport system of nerve terminals may inactivate the action of GABA by reuptake. (Supported by U.S.P.H.S. Grant NS-11310 and Training Grant GM-550).

1464 ANTAGONISM OF THE IN VITRO EFFECTS OF Δ^9-TETRAHYDROCANNABINOL AT CHOLINERGIC JUNCTIONS BY ESERINE. Sheldon H. Roth, Division of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 1N4.

Δ^9-tetrahydrocannabinol (Δ^9THC) is one of the major psychoactive constituents of marihuana. Although its mode of action is unknown, it has been classified as a partial anesthetic, suggesting a combination of "specific" and "non-specific" membrane actions. The central activity of Δ^9THC has been attributed to its anti-cholinergic action at post synaptic receptors, similar to the action of atropine at these sites. Δ^9THC depresses the electrically stimulated contraction of longitudinal strip of guinea pig ileum in the dose range of 10^{-9} to 10^{-6} Molar. This effect is believed to be a result of the inhibition of release of acetylcholine from presynaptic terminals of the myenteric plexus. Contractions produced by exogenous acetylcholine are not reduced by Δ^9THC, providing evidence for a presynaptic site of action. The depressant action of Δ^9THC can be reversed by eserine in a dose related fashion. Pretreatment with eserine also prevents Δ^9THC from exerting its maximum depressant action.

In addition, in the presence of eserine, the release of acetylcholine from longitudinal strips is not reduced by Δ^9THC. Thus, eserine may antagonize the effect of Δ^9THC by functional antagonism, i.e. an excitatory effect, or a competitive antagonism at a presynaptic site.

(Supported by the Medical Research Council of Canada)

1465 EVIDENCE FOR GENERATION OF EXCITATORY POSTSYNAPTIC POTENTIALS BY CURRENT FLOW 'ACROSS' THE MEMBRANE CAPACITANCE AT A PERIPHERAL MAMMALIAN SYNAPSE.
E.M. Silinsky* and G.D.S. Hirst* (SPON: A.F. Boyne) Dept. of Physiol., Monash University, Clayton, Victoria, Australia.

The interaction of excitatory postsynaptic potentials (esps) and inhibitory postsynaptic potentials (isps) was investigated in neurons of the submucous plexus of the guinea-pig small intestine using intracellular recording. The esp (a brief event) and the isp (a long-lasting event) were initiated by electrical stimulation of different presynaptic fibers using 2 pairs of transmural stimulating electrodes. Electrotonic potentials produced by passing current through the recording electrode were used to measure cell input resistance. During the prolonged conductance increase produced by the inhibitory transmitter, the esp recorded at the isp equilibrium potential was less depressed in amplitude than was the electrotonic potential. Part of this depression of the esp may be due to presynaptic inhibition of release of excitatory transmitter by the inhibitory transmitter (e.g. the actual effect of the postsynaptic inhibitory conductance change on the esp amplitude may be even smaller than the observed effect). Nevertheless, the more rapid time course of the esp seen during the inhibitory conductance change suggests that the esp does experience the conductance increase produced by the inhibitory transmitter. It is suggested that although the resistance change underlying the isp may be dramatic, only a small impedance change is actually presented to the brief excitatory synaptic current which flows predominantly 'across' the membrane capacitance. Postsynaptic inhibition in this system may thus be predominantly due to the hyperpolarizing action of the isp rather than the conductance change produced by the inhibitory transmitter.

1466 PRE AND POST SYNAPTIC ACTION OF SUBSTANCE P IN THE HATCHETFISH.
A. Steinacker*, S. M. Highstein. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

The effect of substance P on synaptic transmission at the hatchetfish Mauthner fiber - giant fiber synapse was tested. The data shows both pre and post synaptic effects of substance P at the synapse. Presynaptic effects are most notable at higher drug concentrations (effective dose $\pm 10^{-5}$M) and consists of a reduction in miniature postsynaptic potential (mPSP) and evoked post synaptic potential (PSP) quantal content. The reduction in mPSP and PSP amplitude also seen at this dose level may be of pre or postsynaptic origin. At lower dose levels no change in mPSP or quantal content was found. A reduction in mPSP and PSP amplitudes were the primary effects at this dose level. No change in postsynaptic current - voltage relationships or membrane potential were seen at any peptide concentration used. The peptide concentration and time course of these effects is in the range of previously recorded data. However, the effect of substance P in this system is contrary to previous work in that it acts to reduce synaptic efficacy.

1467 ANALYSIS OF BINDING AND PHOSPHOLIPASE A PROPERTIES OF A β-BUNGAROTOXIN. G. S. Tobias*, M. Donlon*, W. Shain and G. V. Marinetti*. (SPON: D. E. Evans). Neurobiology Dept., AFRRI, Bethesda, MD 20014, and Biochemistry Dept., Medical Center, University of Rochester, Rochester, NY 14620.

β-bungarotoxin (β-Bgt) acts presynaptically to inhibit neuromuscular transmission. We have previously shown that the β-Bgt has the following two properties: (1) ^{125}I-β-bungarotoxin binds to rat brain synaptosomes, and the binding is inhibited by Ca^{++}; and,(2) the toxin has Ca^{++}-dependent phospholipase A (PLA) activity. We have proposed that β-Bgt exerts its effect on synaptic transmission through a sequence of events: first, binding and then PLA activity. We have been investigating several additional aspects of β-Bgt's binding and PLA properties. We have found that cations inhibit ^{125}I-β-bungarotoxin binding to synaptosomes in the following order of inhibitory potency: $Ca^{++} > Ba^{++} > Sr^{++} >> Mg^{++} > K^{+} > Na^{+}$. Binding is also decreased following pretreatment of synaptosomal membranes with trypsin or chymotrypsin. Pretreatment with neuraminidase, concanavalin A, or phospholipases C or D does not alter binding. However, PLA activity from Vipera russelli venom is inhibitory while bee venom PLA is not. Sr^{++} can substitute for the Ca^{++} required by β-Bgt to hydrolyze egg yolk lecithin. But the maximum PLA activity with Sr^{++} is 30% of that with Ca^{++}. Incubation of synaptosomal membranes (3 mg protein) with β-Bgt (20 μg) for 15 minutes at 37°C results in a partial hydrolysis of phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl serine to their respective lyso compounds and fatty acids. These results suggest that β-Bgt binds to a proteinateous site and rapidly hydrolyzes membrane phospholipids. We are currently analyzing the kinetics of the specific lipid changes in synaptosomal membranes due to β-Bgt's PLA activity.

1468 ENERGY UTILIZATION IN THE LOADING OF NOREPINEPHRINE INTO SYNAPTIC VESICLES. Lawrence Toll*, Cameron B. Gundersen, Jr.* and Bruce D. Howard* (SPON: S. Roberts). UCLA Medical School, Los Angeles, CA 90024.

Nerve impulse-induced release of neurotransmitter from nerve endings requires energy. One possible role for energy utilization in synaptic transmission is the loading of synaptic vesicles with transmitter. Others have demonstrated that ATP stimulates loading of catecholamines into vesicles, but the mechanism has not been determined. We have studied the role of ATP in this process by measuring the effects of ATPase inhibitors and other compounds on the in vitro, ATP-dependent binding of [^{3}H]norepinephrine (NE) to a fraction enriched in synaptic vesicles from rat whole brain. The vesicle fraction was preincubated for 5 minutes at 30° and then with 0.3 μM NE for an additional 5 minutes in a phosphate buffer containing 100 mM K^{+}, 10 mM Na^{+}, 1 mM Mg^{2+} and 1 mM ATP. The bound NE was separated from the medium NE by passage through a small column of Sephadex G-25. Under these conditions, 75-100 pmoles of NE were bound per mg of vesicular fraction protein. ATP stimulated binding about 4 fold. Imido-ATP (AMPPNP) could not effectively replace ATP indicating that ATP must be hydrolyzed to stimulate binding of NE. The binding was inhibited by 0.1 μM reserpine, 50 mM oleate, 0.1 mM dinitrophenol and 3 μM tributyl tin, an inhibitor of mitochondrial ATPase. Tributyl tin also inhibited the Ca^{2+}/Mg^{2+} ATPase activity associated with the vesicle fraction while the other compounds at these concentrations did not inhibit the enzyme activity. Mitochondrial ATPase was not involved in the NE binding because oligomycin, another mitochondrial ATPase inhibitor did not affect NE binding. Ouabain also had no effect. These results indicate that the ATP-dependent loading of vesicles is an energy-requiring process and that a Ca^{2+}/Mg^{2+} ATPase is involved.

1469 GABA EFFECTS ON THE DEPRESSION, FREQUENCY FACILITATION AND POST-TETANIC POTENTIATION AT A CHOLINERGIC SYNAPSE IN Aplysia Californica. J.P.Tremblay and G. Plourdes*. Neurobiology Lab. and Dept. Anat., Laval Univ. Quebec, QUE. G1K 7P4.

Monosynaptic, unitary and cholinergic EPSP's produced by minimal stimulation of the right connective were recorded with a 3M KCl electrode from cell R15.

Trains of 100 stimuli at 1/ sec, followed by test pulses at interval ranging from 20 to 200 sec were given every 10 to 30 min (variable in different preparation). At this frequency, in most of the preparation, the EPSP's first decreased in size (depression) and then increased to a sustained facilitated plateau (frequency facilitation). The train was followed by a period of post-tetanic potentiation (PTP) during which EPSP's were even larger than at the facilitated plateau. The PTP duration lasted an average of 20 min under these experimental conditions. At least 2 trains were given before perfusing GABA at concentration ranging from 10^{-4} to 10^{-3}M. GABA reduced significantly the size of every EPSP's obtained during the train and the following PTP period. All the EPSP's were not affected to the same extend so that after 30 to 60 min of perfusion with GABA the depression given by (EPSP1/EPSP2) was reduced, the facilitation (EPSP100/EPSP1) was increased and the PTP (EPSPptp/EPSP100) was reduced. These effects were attributed to a presynaptic action of GABA on this cholinergic synapse because GABA at these concentrations increased only slightly the membrane resistance and did not reduce an ACh potential obtained by iontophoretic application on R15. The results obtained with GABA contrast with those obtained with serotonin and dopamine in the same preparation since GABA could still produce its effects when all Na^+ of the perfusate was replaced by Li^+ whereas the amines did not. (Supported by the Medical Research Council of Canada)

Tissue Culture

1470 A BICLONAL SYNAPSE BETWEEN A NEUROBLASTOMA X GLIOMA HYBRID AND A CLONAL MYOGENIC CELL LINE. C. N. Christian*, P. G. Nelson, J. Peacock, and M. Nirenberg. NIH, Bethesda, MD. 20014 and Dept. of Neurology, Stanford U., Palo Alto, CA. 94305.

Neuroblastoma X glioma hybrid cells NG108-15 form cholinergic synapses with clonal mouse muscle cells G-8. This is the first time synapse formation has been obtained between two clonal cell lines, and provides unique opportunities to study synaptogenesis and the specificity of synaptic connections.

The presynaptic element is the NG108-15 hybrid, known to synthesize, store, and excrete acetylcholine. When differentiated in 1 mM dibutyryl cyclic AMP, the cells extend long ramified processes and are electrically excitable. The hybrid was initially discovered to be synaptically competent when co-cultured with dissociated normal mouse muscle (Nelson et al., Proc. Nat. Acad. Sci., 73:123). In approximately 20 percent of contiguous hybrid-myotube pairs tested by intracellular recording, hybrid action potentials produced in the muscle depolarizing responses of from 1 to 20 mvolts. The probability of following was less than one, and could often be potentiated at stimulation rates of 3 per second. Synaptic transmission was reversibly blocked by curare or the absence of calcium, and irreversibly blocked by α-bungarotoxin. The PSP reversal potential was between -10 and -15 mV.

Iontophoretic application of serotonin or acetylcholine produced a rapidly desensitizing, depolarizing response in the hybrid, which was blocked by curare. The hybrid serotonin response was not dependent on calcium, had a reversal potential between 0 and -10 mV, and thus probably involves a conductance increase to sodium and potassium. Serotonin can elicit transmitter release when applied to the terminals of the NG108-15 on muscle. By noting serotonin induced PSPs in muscle it was determined that a majority of myotubes are innervated in co-cultures.

G-8 is a clonal myogenic cell line obtained from mouse muscle. In vitro it fuses at confluency to form spontaneously contracting myotubes. Both G-8 and normal myotubes had high input resistances (approximately 8 Mohm) and high peak acetylcholine sensitivities (averages of 900 and 1500 mV/nC for G-8 and normal muscle, respectively). Whereas the sensitivity away from "hot spots" in normal myotubes was low, the sensitivity of G-8 myotubes appeared to be uniformly high.

In co-cultures of NG108-15 and G-8, 28 per cent of tested pairs had synaptic connections, with following rates of less than one and PSP amplitudes of from 0.4-8 mvolts. Thus, the hybrid forms immature low efficiency synaptic connections with the G-8 as readily as with primary mouse muscle. The high incidence of functional connections between the hybrid and either muscle type indicates that a cholinergic neuron can synapse with any muscle cell that synthesizes an adequate number of acetylcholine receptors. Therefore, if recognition molecules are required for synapse formation, they appear to be expressed in conjunction with the receptor. Biclonal systems may allow us to separate the variety of determining factors that may be involved in synaptogenesis.

1471 FORMATION OF SPECIFIC SYNAPTIC NETWORKS BETWEEN DORSAL ROOT GANGLIA AND LONGTERM-DEAFFERENTED EXPLANTS OF FETAL MOUSE SPINAL CORD AND MEDULLA. Stanley M. Crain and Edith R. Peterson.* Depts. of Neuroscience and Physiology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

In previous studies we demonstrated that fetal mouse dorsal root ganglion (DRG) neurites can form functional synaptic connections with specific types of target neurons during co-culture with explants of dorsal horn regions of spinal cord or dorsal column nuclei of medulla (Br. Res. 79:145, '74; Science 188:275, '75; Soc. Neurosci., '75).

We have now applied this model system to investigate the degree to which DRG-target sites in CNS explants remain receptive to innervation after long periods of sensory deprivation in culture. Fetal mouse (14-day) dorsal cord strips or medulla cross-sections at the level of the dorsal column nuclei were explanted alone (devoid of meningeal covering), for 1-3 weeks, on collagen-coated coverslips in Maximow slide chambers. Cross-sections of 14-day fetal mouse spinal cord with attached DRGs, or isolated clusters of DRGs, were then positioned about 0.5-1 mm from the deafferented CNS target tissues and the cultures were maintained for an additional 1-5 weeks prior to electrophysiologic analyses. Prior to explantation, a midline section of the cord fragment was made through the dorsal cord and meninges to ensure outgrowth of CNS neurites, including "dorsal column" axons, towards the target explants. NGF (1,000 units/ml) was added to the culture medium after inserting the fetal DRGs to enhance survival of DRG neurons and their neuritic outgrowth. Extracellular recordings were made with Ag-AgCl electrodes via saline-filled micropipettes (3-5 μm tips) and electric stimuli were applied via similar pipettes with 10 μm tips.

Focal DRG stimuli evoked characteristic negative slow-wave potentials in the target regions of the longterm-deafferented CNS explants. These responses arose abruptly after latencies of a few msec and often lasted more than 500 msec, similar to the sensory-evoked dorsal-horn potentials observed in explants of cord with attached DRGs (resembling PAD responses *in situ*). Indirect DRG-evoked cord and medulla discharges mediated via CNS interneurons were generally blocked after introduction of 10^{-3}M GABA, whereas the shorter-latency PAD-like responses were unaffected or enhanced. Functional DRG-innervation of deafferented CNS explants has been successful in 11 cases involving 1-2 week delays before introduction of the DRGs, and 5 with a 3-week lag.

The 14-day fetal mouse spinal cord and medulla explants were "deafferented" prior to or during early stages of synaptogenesis. Although complex synaptic networks develop rapidly in these isolated CNS explants, the present experiments demonstrate that DRG receptor sites on the CNS target neurons remain available for orderly innervation for periods of at least 3 weeks *in vitro*. The sensory-evoked network discharges which develop after DRG innervation have been interpreted to include potentials generated by CNS internuncial axons synapsing on DRG terminals, forming presynaptic inhibitory circuits. This suggests that neurons in our deafferented cord and medulla explants may retain the capacity to sprout new collaterals after the long-delayed arrival of DRG neurites.

This model system will now be extended to problems in CNS reinnervation by determining if specific synaptic networks also form when DRGs are presented to longterm-deafferented dorsal cord or dorsal column nuclei which had already been well innervated *in situ* or in culture. DRG-vacated postsynaptic receptor sites may atrophy after prolonged deafferentation of more mature CNS explants or they may under these conditions become re-occupied by non-specific collateral sprouts from neighboring CNS neurons. (Supported by grants NS-06545, -08770, and -06735 from NINCDS; BMS75-03728 from NSF.)

1472 INNERVATION AND MATURATION OF MOUSE SKELETAL MUSCLE IN CULTURES DEFICIENT IN ORGANOTYPIC NEURAL COMPONENTS. Edith R. Peterson* and Stanley M. Crain (SPON: E.R. Masurovsky). Depts. of Neuroscience and Physiology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

In previous studies of fetal mouse spinal cord-ganglion explants co-cultured with adult or fetal muscle we demonstrated organotypic development and maturation of neuromuscular junctions comparable to mature motor endplates in situ (Exp.Neur. 36:136,'72; Ann.NYAS 183:33,'71; 228:6,'74). The present investigation concerns: 1) the longterm stability of neuromuscular junctions formed by structurally deficient motor nerve fibers -- lacking Schwann cell sheaths, myelin, and terminal Schwann cells; and 2) the capability of nonspecific cord neurons to form functional relationships with muscle. Fetal mouse spinal cord, stripped of meninges and divided into dorsal and ventral strips, was presented adult thigh muscle fibers, ca. 0.5 mm away. All nerve fibers and roughly 1/3 of the muscle, from its origin through the well-ordered motor endplate region, were eliminated to ensure that no Schwann cells are introduced into culture via the muscle explant. About 75% of the muscle regenerates co-cultured with ventral cord matured and could be maintained for more than 3 months in vitro (I.V.), showing stable cross-striations and spontaneous contractions. Characteristic CNS neuritic outgrowth with scattered glial cells formed a bridge between the explants and spread over the regenerating muscle fibers. Branching nerve terminals were observed in the muscle (by silver impregnation) and foci of cholinesterase activity were demonstrated (Karnofsky technique). Vigorous contractions of large groups of muscle fibers could be evoked repeatedly with small single electric stimuli to ventral cord (up to 3 months I.V.), as in co-cultures of muscle with the entire cord-ganglion complex. These contractions could be selectively blocked by d-tubocurarine (1 μg/ml). Most of the dorsal cord strips, while supporting muscle regeneration, did not prevent muscle atrophy by 2-3 wks. No muscle contractions could be detected with large single or tetanic stimuli to dorsal cord explants in this group, although direct stimuli evoked normal contractions. In a few cultures, however, which retained some cross-striated muscle, a few fibers showed contractions in response to 100/sec dorsal cord volleys (3 wks. I.V.), but these responses could only be evoked occasionally after long rest periods.

In comparable co-cultures with 18-day fetal muscle there was similar ventral cord innervation, but also a few dorsal cord cultures (3) in which muscle contractions could be readily evoked by small single cord stimuli and selectively blocked by d-tubocurarine (2-3 wks.). In order to eliminate the possibility of contamination of the dorsal cord strips by occasional motor neurons, the cord was further divided into dorsal, medial and ventral strips. As anticipated, essentially no neurally evoked contractions were detected in preliminary tests of co-cultures with these more restricted dorsal cord tissues (1-2 wks. I.V.), whereas the medial cord explants showed muscle innervation in some cases comparable to ventral cord, in others more labile.

Conclusions:

1) Nerve fibers totally devoid of Schwann cells can establish long-term neuromuscular synapses fully capable of supporting mature structure and normal function of muscle in culture. Electron microscopy is needed, however, to determine whether or not the terminal Schwann cell deficit may result in cytologic abnormalities of the motor endplates.

2) In the absence of ventral motor neurons, nonspecific cord neurons may establish cholinergic neuromuscular synapses, but they appear to involve marked deficits, e.g. labile and less efficient transmission, restricted zones of innervation. These nonspecific cells may include intermediolateral neurons which innervate peripheral sympathetic ganglia or truly intracentral neurons of the medial cord. (Supported by grants NS-08770, -06545, and -06735 from NINCDS; BMS75-13728 from NSF.)

1473 RELEASE OF GLUCOSAMINE-MATERIAL INTO THE CULTURE MEDIUM OF MOUSE NEUROBLASTOMA. V. J. Aloyo, C. Sobhy and W. L. Byrne. Department of Biochemistry, Univ. of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163.

It has been well established that mouse neuroblastoma cells can exist in both a differentiated and undifferentiated state. Previously, the conditions which favored the differentiated state also resulted in a decrease rate of growth. In other cell lines, changes in the growth rate significantly alter cellular physiology and metabolism. Culture conditions which allow the expression of differentiation without altering the growth rate would faciliate the study of those paramaters which are specific to either state.

Mouse neuroblastoma cells clone N18 were grown in Dulbecco's Modified Eagle's Medium supplemented with either 1% or 10% FCS at 37°C in an atmosphere of 10% CO_2 and 90% air. Less than 3% of the cells grown in 10% FCS exhibited a differentiated morphology while 30% to 50% of the cells grown in 1% FCS were differentiated. Under both conditions the generation time was about 19 hours as determined by both cells per flask and protein per flask. Either C^{14}- or H^3 - glucosamine was added to the culture medium (containing either 1% or 10% FCS) for varying times up to 24 hours. The medium was separated from the cells by centrifugation and filtered through a Diaflo UM-10 membrane. The filtrate was fractionated on Sephadex G-10. A comparison of the glucosamine-labeled fractions by paper chromatography or thin layer chromatography (following dansylation) revealed qualitative and quantitative differences between samples derived from the differentiated and undifferentiated conditions.

1474 A METHOD FOR CULTURING ISOLATED, ADULT SKELETAL MUSCLE FIBERS. Anne Bekoff and William J. Betz. Dept. Physiology, Univ. Colorado Sch. Med., Denver, CO 80220.

Flexor muscles of the hind foot (flexor digitorium brevis and f.d. quinti b.) are removed from young adult female Sprague-Dawley rats (150-200 gms) and incubated in Ca-free MEM with 0.3% collagenase (Sigma Type IV) for 2-2.5 hrs at 37°C in a 95% O_2, 5% CO_2, H_2O-saturated atmosphere. Trituration in culture medium (MEM, 10% horse serum, 2% chick embryo extract) releases single fibers, many of which are intact. The short length (ca. 1 mm) of these muscle fibers apparently allows them to withstand the mechanical agitation of trituration.

After plating, a few muscle fibers attach to collagen-coated glass coverslips, but most remain in suspension. The yield of attached fibers is greatly increased by plating them on monolayers of fibroblasts (from 7 day chick embryo hindlimbs) plated 1-2 days earlier.

Electrophysiological measurements of membrane potential, input resistance, and distribution of acetylcholine (ACh) sensitivity as well as light- and electron-microscopic observations show that many fibers prepared in this way remain healthy for at least two weeks, and undergo some of the changes characteristic of denervated muscle. In particular, the excellent visual resolution achieved with isolated fibers permits detailed iontophoretic mapping of ACh sensitivity. End plates, which are often recognizable visually, remain highly sensitive to ACh. In some fibers, a low level of extrajunctional sensitivity is found, which decreases progressively to undetectable levels within 100 μ from the end plate. After 6-7 days in culture, however, the entire extrajunctional surface becomes uniformly sensitive to ACh.

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1475 PGE_1 STIMULATION OF CAMP LEVELS: INHIBITION BY PUTATIVE NEUROTRANSMITTERS IN A NEURONAL SOMATIC CELL HYBRID. James Blosser, Paul R. Myers, and William Shain, AFRRI, Bethesda, Md. 20014.
A somatic cell hybrid (TCX17), a subclone of the embryonic mouse sympathetic ganglion cell X neuroblastoma (N18TG2) cell line NX31, was tested for the ability of neurotransmitter substances to antagonize prostaglandin E_1 (PGE_1) stimulation of cAMP levels. In the presence of phosphodiesterase inhibitor Ro20-1724 (10^{-4}M), cells incubated for 3 minutes in the presence of 10^{-8}M PGE_1 exhibited a 10 fold increase in cAMP levels. The PGE_1 stimulation as well as basal levels of cAMP could be inhibited by coincubation with either 10^{-5}M carbachol, 10^{-6}M norepinephrine, or 10^{-5}M dopamine. In contrast, both serotonin and morphine were ineffective in altering either basal or PGE_1 stimulated increases in cAMP at 10^{-5}M, the latter despite the presence of opiate receptors in this cell line (Blosser et al., Fed. Proc. 34,2797,1975). The carbachol inhibition could be reversed by preincubation with 10^{-8}M of either atropine or scopolamine but not by 10^{-6}M of α-bungarotoxin or d-tubocurarine suggesting the presence of muscarinic receptors. Phentolamine and phenoxybenzamine but not dichloroisoproterenol could reverse norepinephrine inhibition. In addition, isoproterenol (10^{-5}M) could not mimic the norepinephrine inhibition, consistant with an α adrenergic receptor. Chlorpromazine, stelazine and bulbocapnine (all 10^{-6}M) blocked the dopamine inhibition suggesting the presence of a dopamine receptor. Electrophysiologically, dopamine elicits a depolarizing response in TCX17 (Myers et al., Neurosciences Ab. 994, 1975). Thus the possibility exists that a neurotransmitter which elicits a conductance change can also modulate PGE_1 alterations in cAMP levels in this cell line.

1476 EARLY SIGNS OF TRANSMITTER RELEASE AT NEUROMUSCULAR JUNCTIONS DEVELOPING IN CULTURE. S. A. Cohen* (SPON: J. H. Neale). NIH, Bethesda, MD. 20014
Within 24 hours after adding explants of chick embryonic spinal cord to already established chick pectoral muscle cell cultures relative peaks ("hot spots") of acetycholine (ACh) sensitivity have been reported located precisely at transmitter release sites (Cohen and Fischbach, Fed. Proc., 1975). Can the ingrowing neurites also release neurotransmitter over muscle membrane that is unspecialized with respect to ACh sensitivity? Since a quantum of ACh released at hot spots (1000-10,000 mV/nC) was found to generate a 0.5-2.0 mV synaptic potential, if it were released instead over non hot spot ("background") regions of sensitivity (100-300 mV/nC) the response would be too small to discern from recording system noise (~200 μV) with the methods previously used. Experiments were done, therefore, using signa averaging techniques to assay for such transmitter release. Cultures were visualized and studied with Nomarksi differential interference contrast optics a few minutes to a few hours after outgrowing neurites from spinal cor explants first contacted myotubes. Intracellular microelectrode recordings were made from the muscle fibers while stimulating contacting nerve processes with extracellular micropipettes. In many cases 25-100 μV synaptic potentials were discriminated by summing the responses to 32-256 successive nerve shocks. Bath perfusion or local application of d-tubocurarine reversibly blocked the responses. Thus, the synaptic potentials appear to be cholinergic, and their small amplitude is not due to their originating from a site remote from the recording electrode. To determine whether the small size of the synaptic potentials was caused by a presynaptic event such as decreased quantum size or a postsynaptic one such as low receptor density, ACh sensitivity maps were made. Where the full extent of the neurites could be visualized overlying the myotubes the subneural muscle membrane ha background levels of chemosensitivity (100-500 mV/nC). Thus, the low receptor density could account entirely for the small amplitude synaptic responses. ACh receptor accumulation, therefore, is not a necessary condition for presynaptic neurotransmitter release. The experiments suggest that ACh secretion from neurites over muscle membrane with low chemosensitivity is a stage of synapse development preceding release at hot spots.

1477 A NEW TECHNIQUE FOR THE PRIMARY CULTURE OF RAT BRAIN ASTROCYTE-LIKE CELLS: MORPHOLOGY AND HISTOCHEMISTRY. Craig J. Cummins,* Laboratory of Neurochemistry, M.H.R.I., and the Neuroscience Program, and Roy A. Glover,* Department of Anatomy (SPON: L. T. Rutledge), The University of Michigan, Ann Arbor, Michigan 48109.

Astrocyte-like cells were cultured from 3-4 day old rat forebrains. When cells derived from tryptic digested brains are plated at a density of 2 x 10^2 cells/cm^2, the cells are confluent in 12-14 days. Morphologically, the cells are multipolar, possess an oval nucleus, and often two or more nucleoli. The cells have a fine agranular cytoplasm.

When these cells were stained with glial-specific stains, such as the Mallory phosphotungstic acid hematoxylin, or the Holzer glial stain, confluent cells stain in a manner characteristic for astrocytes *in vivo*. Confluent cells are rich in glycogen granules.

When cells are reacted to demonstrate lactic, malic, isocitrate, succinate, or glutamic dehydrogenases, by a modification of the prominent nitro BT technique, granules are diffusely spread throughout the cytoplasm, and throughout the fine interconnecting processes as well.

1478 IN VITRO MIGRATION AND PROLIFERATION OF CELLS FROM ISOLATED MOUSE CEREBRAL CAPILLARIES. L.E. DeBault, L.E. Kahn* and P.A. Cancilla* Dept. Path., U. of Iowa, and Vet. Admin. Hosp., Iowa City, IA 52242.

Microvessels isolated from mouse cerebrum were used as the source material for derivation of brain endothelium in culture. The microvessels were isolated by a modification of a mechanical dispersion and filtration technique described by Brendl *et al.* (Sci. 185:953, 1974). A capillary fraction collected by selective adhesion to a substratum was cultured in Lewis Medium (Sci. 181:453, 1973) containing 30% FCS for the first 7 days and 20% thereafter. Capillaries so isolated were rich in gamma glutamyl transpeptidase and were morphologically intact at the EM level. After 5-7 days in culture the cells of the isolated capillaries began to show expansion of the nucleus and an increase in cell size. This characteristic change was followed by the migration of presumptive endothelial cells from the ends of the isolated capillaries. By 14-21 days, cell proliferation had begun and was followed by monolayer formation. At the light microscope level these mouse brain endothelial cells were morphologically similar in culture to endothelial cells derived from human umbilical vein by Jaffe *et al.* (J. Clin. Invest. 52:2745, 1973). A second cell type was seen migrating from these capillaries which had the cytological characteristics of smooth muscle cells. Current work is focused on the detailed characterization of these cells and will include studies on factor VIII antigen, gamma glutamyl transpeptidase activity, and ultrastructure. (NHLI Grant # 14230-06 and VA research grant #584-1277.01 supported this study.)

1479 CELL MIGRATION AND CELL-CELL INTERACTIONS IN PRIMARY CULTURES OF EMBRYONIC MOUSE CEREBELLUM. M.E. Hatten*, E. Trenkner* and R.L. Sidman. Dept. of Neuropath., Harvard Medical School, Boston, MA. 02115.

The development of the mammalian cerebellum involves in part the proper migration and interaction of immature neurons. We report here the establishment of primary cultures from embryonic and neonatal mouse cerebellum, designed to study cell surface components pertinent both to migration and to cell-cell interactions. When plated at high cell density, embryonic cerebellar cells assemble into reproducible patterns. A polylysine-treated tissue culture substratum is required for the survival of embryonic day 13 (E13) cerebellar cells. This result, as well as the response to various sera, including dilipidated sera, is in contrast to that obtained with cultures of early postnatal cerebellum.

With lectins as probes for carbohydrate-containing surface macromolecules, cells (harvested separately from embryonic cerebellum, midbrain, medulla, spinal cord and cerebral cortex) were tested for agglutination with Concanavalin A, wheat germ agglutinin, ricinus communis, lens culinaris, lotus, wysteria and soybean agglutinin. Different regions of the E13 brain were found to agglutinate with different lectins, indicating probable differences in cell surface carbohydrates.

Embryonic cells tend to migrate out of the aggregates onto the culture substratum as a wave. This result contrasts with that obtained with either postnatal cerebellar cells or embryonic midbrain cells where no migration is observed. Surfaces derivatized with particular carbohydrates are currently being tested for their efficacy as substrates for differential cell migration.

1480 SYNTHESIS AND RELEASE OF ACETYLCHOLINE IN CHOLINERGIC MOUSE NEUROBLASTOMA CLONES. A. C. Kato*, P. Lefresne*, Y. B. Netter*, J. P. Ternaux*, J. C. Beaujouan*, J. Glowinski* and F. Gros. College de France, Paris, France (SPON: K. Krnjevic).

One class of mouse C-1300 neuroblastoma has been defined as "cholinergic" since it contains a high level of choline acetyltransferase (ChAc) and is free of other neurotransmitter-synthesizing enzymes. The clones which have so far been analyzed for the transport of choline were found to be devoid of the specific uptake system characteristic of cholinergic nerve terminals. Therefore it has been questioned to what extent the presence of ChAc is a sufficient marker of a cholinergic phenotype. In the present report we show (i) that high levels of acetylcholine (ACh) exist in two cholinergic clones NS 20 and C 15, (ii) that ACh can be formed from glucose and acetate in these two clones, (iii) that choline can stimulate the rate of ACh synthesis from both glucose and acetate in the NS 20 and C 15, (iv) that choline can increase the influx of acetate in the cholinergic clone NS 20 but not in the adrenergic clone NIE 115 nor in the non-specific clone Cl 22, and (v) that electrical stimulation but not high K^+ can evoke the release of ACh in the NS 20 neuroblastoma.

1481 ELECTRICAL ACTIVITY OF CONTRACTING MYOCARDIAL CELLS IN TISSUE CULTURE. M. Kitzes*, G. Twiggs* and M. W. Berns* (SPON: R. K. Josephson). Dept. Developmental and Cell Biology, Univ. of Calif., Irvine, CA 92717.

The intracellular study of neonatal rat (1-2 day old) ventricular cells in tissue culture shows that contracting myocardial cells exhibit an array of different patterns of spontaneous electrical activity. Variability between cells is evident in the range of their membrane potential (40 mV to 90 mV) and in the shape and amplitude of the spike. Rhythmically contracting cells can show characteristic pacemaker potentials and/or "prepotentials" or neither. Although interspike intervals are remarkably constant in a great proportion of the cells, this is not always the case. Some cells show a cyclic modulation of spike amplitude and frequency; this pattern remained unchanged for the duration of the approximately 20 min. recording periods. There are also a number of "abnormal" kinds of potentials, such as the presence of a notch in the depolarizing phase of the action potential or as is evident in cells with irregular beats. Fibrillating cells typically exhibit asynchronic fluctuating membrane activity.

Laser microirradiation of preselected cells alters their electrical activity. Furthermore, a similar sustained depolarization with gradual repolarization to preirradiation level can be evoked in some cells by irradiation of another cell, several cells apart. (Supported by grants NIH HL 15740, GM 22754, and NSF GB 43527.)

1482 MEMBRANE CHARACTERIZATION OF A CLONAL HUMAN NEUROBLASTOMA, SK-N-SH. T. Kuramoto*, R. Perez-Polo, K. Werrbach* and B. Haber. The Marine Biomedical Inst., Dept. of Neurology & Dept. of Human Biol. Chem. & Genetics, UTMB, Galveston 77550; Dept. of Zoology, Univ. of Texas, Austin 78712.

The SK-N-SH human neuroblastoma was cultured and cloned by Dr. June Biedler, The Sloan Kettering Institute. This cell line is nearly diploid, has high levels of dopamine β-hydroxylase and tyrosine hydroxylase, and no choline acetyltransferase activity. Under standard tissue culture conditions, as used in these experiments, less than one percent of these cells show morphological differentiation (process formation). The electrical properties were studied by standard intracellular recording techniques; the average resting membrane potential was -21 ± 11 mV, with few cells showing membrane potentials greater than -40 mV. In response to current injection, a variety of graded responses with a relatively slow rise time were observed. In some cells only delayed rectification was observed. In no instance did current injection result in a characteristic action potential. The iontophoretic application of acetylcholine to the cell soma produced depolarizing responses, which were dependent on the membrane potential. An analogous method for determining electrical excitability is to measure Na^{22} influx in the presence and absence of a depolarizing agent, veratridine (0.1 mM). In such experiments, the influx of Na^{22} in SK-N-SH cells was not altered by veratridine in contrast to the N18 murine neuroblastoma cells, which are electrically excitable. Taken together, these data suggest that the morphologically undifferentiated human neuroblastoma cells are relatively inexcitable. It is therefore possible that morpohological differentiation is concomittant with the expression of Na^+ channels and spike generation.

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1483 EXPLANT CULTURE OF ADULT GOLDFISH RETINA FOLLOWING OPTIC NERVE CRUSH-AN IN VITRO MODEL FOR THE STUDY OF CNS REGENERATION. G.E. Landreth and B.W. Agranoff, Neuroscience Laboratory,University of Michigan, Ann Arbor, Michigan 48109.

Explantation of Xenopus retina (Agranoff, Field and Gaze, Brain Res., in press) results in outgrowth of neuritic and non-neuritic elements. Conditions for successful culture of adult goldfish of retina have now been determined. Uniform explants (600 μm square) are obtained by using a McIlwain tissue chopper. Explants are maintained in a modified L-15 medium in air at 22°. Growth was seen on collagen gels or polyornithine-treated glass. The retina remains morphologically intact even after two weeks in culture. As in Xenopus, optic nerve section several days prior to explantation results in a vigorous neuritic outgrowth in culture. The neurites are beaded and are characterized by active growth cones with microspikes at the advancing tip. Normal (unoperated) retinas eventually develop neuritic growth after several days in culture, but less than in prior crush retinas. Since retinas whose optic nerve had not been previously crushed do eventually show neuritic outgrowth, it appears that retinal events leading to initiation of regeneration can occur in vitro. In a series of animals the right caudal tectum was ablated,denervating the left nasal retina. Ten days later the retina was cut into nasal and temporal hemi-retina. Explants from the denervated (nasal) area of the retina showed neuritic growth characteristic of prior-crush retina, while temporal retinal explants exhibited growth similar to control (unoperated) tissue. Thus it appears that the observed outgrowth is an intrinsic property of the cells involved and is not mediated by diffusable factors. Supported by NIMH 12506 and BMS 75-03810 from NSF. G.L. was a trainee of MH 14279.

1484 CHARACTERISTICS OF GLIAL AMINO ACID TRANSPORT AND EFFECTS OF GLIAL PROLIFERATION. William J. Logan. Dept. Neurol., Sch. Med., Univ. Va. Charlottesville, Va. 22901.

Glia interact with the extracellular fluid through membrane transport processes which may contribute to the maintenance of the microenvironment of the central nervous system (CNS). In gliosis and other glial reactions with altered membrane morphology and chemistry there may be related changes in these transport mechanisms. This was investigated by evaluating the transport of 12 amino acids by two glial cell lines in culture and in a model of glial proliferation.

The profiles of amino acid accumulation in C-6 and C-2_1 were similar. Uptake of glutamic acid was greatest and that of gamma-aminobutyric acid least in both cell types. Glial proliferation was induced in C-6 glia by increasing the concentration of fetal bovine serum in the culture medium from 5% to 25%. This resulted in altered cell and culture morphology and selective increases in amino acid uptake. Leucine, valine and phenylalanine uptakes increased five-fold. Uptake of aspartic and glutamic acids, lysine and serine more than doubled, but that of the other amino acids showed no change. The individual transports were distinct in their response to substrate inhibition and to sodium omission. Uptake of the putative neurotransmitters did not manifest the marked sodium dependence seen in CNS preparations.

It is concluded that glial cells have characteristic membrane processes for amino acid transport which can interact with the microenvironment. These systems undergo profound but selective changes during glial proliferation induced by serum. Similar alterations may occur during gliosis, resulting in disturbance of the microenvironment and secondarily of neural function.

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1485 INDUCTION OF RNA SYNTHESIS BY NOREPINEPHRINE IN PRIMARY DISSOCIATED RAT BRAIN CULTURES. R. Maurer and A. Grieder*. Sandoz Ltd., Medical and Biological Research, CH-4002 Basel, Switzerland.

Primary dissociated cultures from 17- or 20-day old fetal rat brain (17FRB or 20FRB) differ both in their morphological appearance and biochemical responsiveness. Whereas many phase contrast bright single neurons and clusters of neuronal cells can be observed in 17FRB cultures, such cells are almost absent in 20FRB cultures. Stimulation of cultures with norepinephrine (NE) results in a concentration dependent increase in the rate of uridine incorporation into trichloroacetic acid precipitable material. A maximal increase is obtained during a 60 min. pulse directly after addition of NE at a concentration of 10^{-6} - 10^{-5} M. In 20FRB cultures, the incorporation of uridine is almost twice that in untreated controls (97 $\pm$ 6.5%, 10^{-5} M NE); a much smaller response (14.5 $\pm$ 3.6%, 10^{-5} M NE) is observed in 17FRB cultures. Additional experiments allow the conclusion that enhancement of uridine uptake is not due to pool fluctuations or transport changes, but reflects a true increase in RNA synthesis. The data obtained show differences in the cellular population in cultures depending on the age of the foetuses. Since a minority of cells in 20FRB cultures can be definitely characterized as differentiated neurons, it seems most likely that the biochemical events after NE stimulation do not take place in neuronal but in glial cells.

1486 INFLUENCE OF DIFFERENTIATING LIMB TISSUE ON NEURITIC OUTGROWTH FROM SPINAL CORD CULTURES. E.D. Pollack, J. Koves* and V. Liebig*. Ill. Inst. Develop. Disabil., Chicago, IL. 60608.

Differentiating limb tissue appears to affect nerve fiber outgrowth from larval frog spinal cord in tissue culture. Cross-sectional spinal cord explants from Rana pipiens larvae at stages V and IX (Taylor-Kollros Stages, Anat. Rec.,'46) were cultured in serum-free medium on collagen substratum (Pollack and Koves, TCA Manual, '75). Epidermis-free hindlimb explants at stage V or IX were introduced to the culture at either the initial cord explanting or after the establishment of neuritic outgrowth (9-13 days *in vitro*). When a stage V limb explant (dense mesenchyme) was presented to stage V cords initially, an enhancement of neuritic outgrowth and fiber orientation toward the limb occurred, in contrast to limb-free cultures. Less growth enhancement was demonstrated in the presence of the more differentiated stage IX limb, although there was pronounced fiber orientation. Stage IX cords, cultured with stage V or IX limb explants exhibited reduced neurite response, albeit enhanced over stage IX controls. The addition of the limb tissue after the establishment of neuritic outgrowth resulted in greater growth enhancement and complexity of neuritic patterns than when cord and limb were explanted simultaneously. Re-orientation of neurites toward the limb tissue was a frequent occurrence. Additionally, there was a significant increase in survival time of these neurites in the serum-free medium over control situations. Inclusion of limb muscle fragments from climactic larvae resulted in enhanced outgrowth from stage V cords during the phase of muscle regeneration, and prolonged survival time of both stage V and IX cord explants. Thus, the state of differentiation of the limb may influence spinal cord nerve fiber growth and maintenance *in vitro* and in the *in situ* corollary.

1487 FLUORESCENT HISTOCHEMICAL AND MORPHOLOGICAL CHARACTERISTICS OF LOCUS CERULEUS CELLS IN CULTURE. M. Schlumpf, W. J. Shoemaker, and F. E. Bloom. Lab. Neuropharm., NIMH, St. Eliz. Hosp., Washington, D.C. 20032.

Explants of embryonic rat brain-stem regions can be maintained in culture for up to 5 weeks. The norepinephrine-containing cells of the rat locus ceruleus synthesize norepinephrine in vitro. (Schlumpf and Shoemaker, Neuroscience Abst., 1975, pg. 811). Most of the synthesized catecholamines (CA) are released into the medium with very little stored; this situation requires a modified procedure for CA histofluorescence of the cultured cells. Monoamine fluorescence of these cells was achieved after high doses (0.5×10^{-3} M) of pargyline and multiple doses (0.5-1 mg) of ascorbic acid. The cultures were superfused with cold, slightly hypertonic formaldehyde solution (5%), air dried, and exposed to formaldehyde vapor (50% humidity) for 1 hour. In some cultures, only a few scattered cells could be identified while other cultures of the same series reveal clusters of fluorescent cells with long processes, very similar in appearance to the locus ceruleus cell group in situ. Perfusing the embryonic rat brain with hypertonic formaldehyde solution also resulted in consistently bright monoamine histofluorescence; this concomitant finding could indicate that certain immature CA-containing cells in the brain and locus ceruleus cells maintained in vitro share common characteristics. The ultrastructure of locus ceruleus cultures, 2 to 4 weeks in vitro, discloses many specialized contacts both on processes and cell bodies. By using permanganate, the number and characteristics of CA-containing terminal contacts can be evaluated. Hence, the combination of light, electron and fluorescence microscopy provides a comprehensive picture of the morphology of cultured locus ceruleus neurons. Such a view is a necessary requirement for further studies of the physiological and pharmacological assessment of cultured neuronal systems.

1488 ELECTROPHYSIOLOGICAL PROPERTIES OF SMOOTH MUSCLE IN DISSOCIATED CELL CULTURE. C. Nelson Sinback* and William Shain. Dept. of Neurobiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20034.

Human oviduct smooth muscle cells grown for as long as 1.5 years in vitro express electrophysiological properties of smooth muscle in vivo. Single isolated cells and cells in contact with neighboring cells were impaled with 1 or 2 microelectrodes. The mean resting membrane potential was 42±9 mV (n=87). Input resistances were calculated from electrotonic potentials due to current injected via microelectrodes. In 10 isolated single cells the input resistance was 54±28 MΩ. Using photographs to calculate cell surface area, the specific resistance was 41±8 $k\Omega\text{-cm}^2$. The time constant of the electotonic potential was 96±31 msec. These values agree with the predictions of single cell properties (Tomita, 1970). The input resistance of cells in contact with neighboring cells was 18±14 MΩ (n=72). Current passed into either of two connected cells always elicited electotonic potentials in both cells (n=10). Thus, current spread across cell contacts accounted for lower input resistance of connected cells. In a subpopulation of cells active depolarizing potentials were elicited by depolarization to a threshold potential or by relaease from hyperpolarization. Active response amplitude and duration at half-amplitude were 10 mV and 200 msec, respectively. Although spontaneous active responses were not seen, pacemaker potentials were elicited by 10 mM $BaCl_2$. Muscle excitability depended on calcium since 2 mM EGTA or 10 mM $CoCl_2$ abolished the active response.

1489 CORRELATION OF AMINO ACID UPTAKE IN PIA ARACHNOID EXPLANTS WITH CULTURES OF FIBROBLASTS. M. Spatz, M. R. Murray and I. Klatzo. NIH, Bethesda, MD. 20014.

The radiolabeled uptake of the metabolizable Isoleucine and the non-metabolizable Cycloleucine were studied in 14 day old pia arachnoid explants and cultures of skin fibroblasts obtained from newborn rats. The methods used were those described for the uptake of glucose analogues by pia arachnoid (Spatz et al., Brain Res. 100: 710-715, 1975). The Isoleucine uptake of pia arachnoid explants was lower than in the fibroblastic cultures. The concentrations of Isoleucine in the pia arachnoid never exceeded the concentrations of the medium, while the fibroblastic culture Isoleucine uptake was 12 times higher than the concentrations in the incubating medium. In both types of cultures, the uptake was saturable and 70-90% of the uptake could be inhibited by 1 mM of unlabeled Isoleucine. The uptake of Cycloleucine was saturable also and so far has been determined in the pia arachnoid explants only. The radiolabeled uptake of this nonmetabolizable amino acid can be inhibited by unlabeled Cycloleucine, Isoleucine, L-leucine, but not by D-leucine in these cultures. The preliminary data suggest that the uptake of the amino acids studied takes place by carrier mediated transport in pia arachnoid, as well as in the fibroblasts. However, the pia arachnoid is distinctly different from the fibroblasts in being unable to accumulate the amino acid Isoleucine and therefore may represent the responsible site for the regulation of amino acid content in the central nervous system.

1490 HISTOGENESIS OF NORMAL AND MUTANT MOUSE CEREBELLAR CELLS IN A MICRO CULTURE SYSTEM. E. Trenkner*, M.E. Hatten* and R.L. Sidman (SPON: L.Eisenberg) Dept. of Neuropath., Harvard Medical School, Boston, MA 02115.

A micro tissue culture system has been developed using mouse cerebellar cells. Trypsinized single cell suspensions assemble in vitro into reproducible three dimensional patterns of integrated and migrating cells. The patterns formed suggest expression of certain cell interactions characteristic of development in vivo, and can be changed or abolished through the use of different sera in the culture medium. E.g., cells grown in horse serum have a greater affinity for each other than do cells grown in fetal calf serum. Serum properties are not changed by ether extraction.

After 16-18 hours in culture, reaggregates of various sizes had formed and adhered to the surface of the culture well. Aggregates contained granule cells in packed clusters, interneurons of the molecular layer, large neurons and glial cells. Reaggregates were interconnected by fiber bundles attached to non-neuronal cells along the bottom of the well, and by cable-like structures suspended between the reaggregates. Migrating granule cells were identified along both types of fiber bundles. Synapse formation (axo-axonal, axosomatic) began at 72 hours and continued for at least seven days.

Cerebellar cells of postnatal day 6-7 weaver mice do not form connecting fiber bundles under normal culture conditions and relatively few granule cells survive. Weaver cerebellar cells do form fiber bundles and granule cells survived longer in cultures fed with ether-extracted serum. Further, serum of weaver mice contains twice the cholesterol and lipid concentration of their normal littermates. Possible relevance of these findings to expression of the weaver mutation is under study.

1491 AN IN VITRO MODEL OF GLIOBLAST MATURATION. David E. Turriff*, Shuang S. Troy* and Ramon Lim. Depts. of Surgery(Neurosurgery) and Biochemistry and Brain Research Institute, University of Chicago, Chicago, IL. 60637

Exposure of cultured glioblasts to a protein factor(MW 350,000) from adult brain results in morphological and biochemical changes indicative of glial maturation. The appearance of the cells is changed by the factor from an epithelial-like cell monolayer to an interconnecting cell net of multipolar glia. Maximum morphological change occurs at four days and is characterized by extensive process outgrowth. This is accompanied by increases in cyclic adenosine monophosphate, S-100 protein and glycerol phosphate dehydrogenase specific activity. Lactate dehydrogenase specific activity does not increase, however, indicating that the response to stimulation shows some selectivity. Ultrastructurally, the desmosomal cell junctions found in the unstimulated glioblasts are replaced by gap junctions in the mature glia.

The purification scheme for the active protein factor has resulted in over a thousand-fold enhancement of specific activity. Furthermore, the large molecular weight protein can be dissociated by n-butanol extraction into two smaller components: a non-dialyzable, heat labile protein and a slowly dialyzable, heat stable molecule. Both components are necessary for activity.(Supported by NIH grants NS-09228, CA-14599, Ns-07376)

1492 HYPOTHALAMIC CELLS IN PRIMARY MONOLAYER CULTURE. Dennis Vaccaro, Anne Messer and Susan E. Leeman, Depts. of Physiology and Neuropathology, Harvard University Medical School, Boston, MA. 02115.

Hypothalami from 18 day-old embryonic rats are dissected, pooled and incubated in a calcium-free Tyrodes solution containing 0.1% papain and 0.1% deoxyribonuclease. These cells are dispersed by trituration and approximately $8X10^5$ cells are plated per 35 mm dish on a substratum of either glass or plastic. Initial plating medium is Minimal Essential Medium, 10% fetal calf serum, 0.3 M glucose and 15 units/ml penicillin-streptomycin with or without the mitotic inhibitor cytosine arabinoside (AraC, $2X10^{-6}M$). Cells are then fed every third day with medium containing 10% heat-inactivated horse, instead of fetal calf, serum. The cultures are incubated at 35.5^oC, 100% humidity and 5% CO_2.

Immediately after dissociation, there are primarily single, rounded cells with occasional small clumps. At 3 days in vitro (DIV) AraC treated and untreated cultures are morphologically indistinguishable, each having many neurons with processes of intermediate length and a non-confluent background of non-neuronal cells. After 7 DIV, untreated cultures are densely confluent with many foci of ciliated ependymal cells. Only short neuronal processes are visible. AraC treated cultures are less densely confluent and contain neurons that have elaborated many long, multi-branched processes. Untreated cultures have up to twice the protein content of AraC treated cultures. Both types remain viable for as long as ninety days.

The existence of neurons in these cultures has been confirmed by intracellular recording. In two cultures examined, cells which exhibited neuronal morphology also demonstrated action potentials and received spontaneous synaptic input (excitatory and/or inhibitory). Physiological and biochemical studies are now in process in order to demonstrate other hypothalamic functions in culture. (Grant No. 5 ROI AM 16510).

1493 TYROSINE HYDROXYLASE AND CATECHOLAMINE SYNTHESIS IN LONG TERM CULTURES OF DISSOCIATED BOVINE ADRENAL CHROMAFFIN CELLS. J. Waymire*, C. Cotman, E. Nylen*, and E. Sandoval*(SPON: N.M. Weinberger). Dept. of Psychobiology, University of California, Irvine, CA 92717.

In an effort to establish a system to study the molecular aspects of the regulation of catecholamine biosynthesis and release we have investigated the optimal conditions for long term tissue culture of dissociated bovine adrenal medulla cells. The criteria used to evaluate the physiological competence of these cells was: 1) levels of tyrosine hydroxylase, 2) capacity to synthesize catecholamines, and 3) continued presence of chromaffin vesicles containing dense cores. Based on these criteria, adrenal medulla cells which have been dissociated by digestion with 0.1% collagenase and 0.1% hyaluronadase can be maintained in culture for months if grown as small aggregates of cells in a suspension culture. Cells survive best in 10% CO_2 at 37°C in Ham's F-12 medium containing ascorbate and 10% newborn calf serum. Under these conditions the aggregated chromaffin cells maintain tyrosine hydroxylase levels similar to the freshly dissociated cells (49 pmole DOPA formed per minute per 10^6 cells), synthesize labeled catecholamines from labeled tyrosine, and possess dense core chromaffin vesicles. When cultured as monolayers the cells do not retain these characteristics. Initial studies indicate that this culture preparation may be useful for the analysis of a number of problems related to the molecular disposition of catecholamines. As an example these cells have been found to regulate tyrosine hydroxylase levels in response to increased tyrosine concentrations in the culture medium and show Ca^{++} dependent stimulus secretion coupling of endogenous catecholamines in response to either high K^+ or acetylcholine.

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1494 MORPHOLOGIC AND BIOCHEMICAL OBSERVATIONS IN CHICK DORSAL ROOT GANGLION (D.R.G.) CULTURES TREATED WITH DELTA-AMINO LEVULENIC ACID (δ-ALA) AND LEAD. W. O. Whetsell, Jr.*, S. Sassa*, A. Kappas*. (SPON: T. S. Elizan). Dept. of Neurology, Mt. Sinai School of Medicine, and Rockefeller Univ., New York City.

Groups of mature (3-4 week) sibling cultures of chick D.R.G. were treated with different levels of δ-ALA (0.5mM to 10mM) in standard feeding medium for periods from 12 hours to 144 hours. Living cultures were studied at specific intervals by light and fluorescence microscopy. Other groups of cultures, treated identically were analyzed biochemically for presence of porphyrins at 12, 24, 36, 48 and 144 hours.

Though no observable morphologic change occurred in the living cultures (by light microscopy), a highly unstable orange autofluorescence developed in some cellular components of living cultures (by fluorescence microscopy), reaching an apparent maximum at about 48 hours. Cells of the configuration and distribution of fibroblasts, Schwann cells, and macrophages, showed bright fluorescence while such fluorescence could not be seen in cells identified as neurons. Biochemical analysis of whole cultures showed that significant levels of porphyrins accumulated in the cultures in less than 12 hours and reached a peak between 24 and 48 hours.

Addition of lead acetate (range: 0.01mM to 0.5mM) to cultures treated with δ-ALA produced a 30-40% reduction of porphyrin levels when compared biochemically to sibling cultures treated with δ-ALA alone.

Results demonstrate that certain cellular components of mature, organized cultures of chick dorsal root ganglion possess a porphyrin biosynthetic pathway and that this pathway may be subject to inhibition by lead.

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1495 STUDIES OF SYNAPTIC CONNECTIONS IN CEREBELLAR TISSUE CULTURES.
J.M. Wojtowicz, K.C.Marshall and W.J. Hendelman.Depts. of Physiology & Anatomy, University of Ottawa, Ottawa, Ont. K1N 9A9.

Cultures are explanted from newborn mice and incubated in Maximow chambers at 35°C for 3-4 weeks. These mature cultures are placed in a chamber which is fixed to the stage of an inverted microscope, permitting observation of cultures during the electrophysiological experiments. The chamber is constantly perfused with Earle's balanced salt solution (temp. 35-37°C, pH 7.3-7.4). Single or multibarelled micropipettes are used for extracellular recordings and platinum or tungsten needles for the stimulation. Electrical stimulation of the cortical region in these cultures produces an inhibition of cells in the region of deep cerebellar nuclei (DN). The inhibition can be seen as a depression of the spontaneous or glutamate induced spiking activity. In some cases the inhibition is followed by late excitation, in others by repetitive periods of excitation and inhibition lasting up to one second. The responses are quite similar to those seen by Ito et al (Exp.Brain Res. 10, 64,1970) in cats and are thought to be mediated by axons of Purkinje neurons. Iontophoretic pulses of γ-amino butyric acid gave a similar depression of DN cells, and both were blocked by 4-8 μM of bicuculline. The stimulation of DN usually produces single spike responses of cortical cells (presumably Purkinje cells) which are compatible with the mossy fiber type of connections. The presence of these connections is supported by the observation that cells from DN can be excited antidromically by stimulation of the cortex. Such reciprocal synaptic connections between cortex and DN might be responsible for the observed repetitive discharges of DN cells.

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Trophic Functions

1496 NEUROTROPHIC CONTROL OF SKELETAL MUSCLES IN NORMAL AND HIBERNATING GROUND SQUIRRELS. S.S. Deshpande*[1], E.X. Albuquerque[1] and L. Guth[2]. Dept. Pharmacol. Exp. Ther.[1] and Dept. Anat.[2], Sch. of Med., Univ. of Maryland, Baltimore, MD. 21201.

In rat skeletal muscle the first sign of neurotrophic regulatory loss after denervation is a membrane depolarization of 15-20 mV. Although the membrane potential (MP) of normal skeletal muscle appears to be maintained by a neurotrophic mechanism(s), factors other than neurotrophic regulation (such as the presence of neuromuscular transmission and muscle activity) have also been proposed for the maintenance of MP. The precise factor(s) controlling MP can be more clearly resolved by studying skeletal muscles of animals during deep hibernation when complete and prolonged muscle inactivity exists. The membrane properties of *fast* extensor digitorum longus and *slow* soleus muscles of ground squirrels (*Citellus tridecemlineatus*) hibernating at 5-7°C were studied *in vitro* with conventional microelectrode techniques at bath temperatures of 5, 15 and 21°C. Hibernation does not alter the MP of either muscle (-77.0 ± 0.4 mV) but when denervated during hibernation the MP decreased 10-15 mV. These findings show that muscle activity is not necessary for the maintenance of the normal MP and that even at low body temperatures (5-7°C), the muscle membrane will depolarize after denervation. In nonhibernating squirrels, spontaneous and evoked transmitter release disappears within 15 days after denervation. In hibernating squirrels, however, these muscles display miniature endplate potentials with amplitudes of 0.8-1.0 mV at a frequency of 0.2-0.3 sec^{-1} for at least 30 days after denervation; indirectly elicited action potentials could also be evoked with rates of rise of 436 V/sec. This indicates that the depolarization observed in denervated skeletal muscles is unrelated to transmitter release. There is also no extrajunctional acetylcholine (ACh) sensitivity in either innervated or denervated skeletal muscle of hibernating squirrels. In addition, chronically denervated muscles of both hibernating and nonhibernating squirrels were as sensitive to the action of tetrodotoxin (3 μM) as innervated muscles. The study of muscles from hibernating animals also provides important clues to the subject of physiological adaptation and neurotrophic regulatory mechanisms. (Supported in part by USPHS grants NS-12063 and NS-12847 and by the Paralyzed Veterans of America.)

1497 "TROPHIC" CONTROL OF MUSCLE CHOLINESTERASE. Norman A. Ranish and Wolf-D. Dettbarn, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

Denervation of muscle produces a significant loss of muscle end-plate cholinesterase activity. In addition, only reinnervation of a denervated muscle can affect a return of enzyme activity to normal levels. Since it has been assumed that the mechanism underlying this phenomenon is a reflection of the "trophic" effect of nerve on muscle, studies have been directed toward examining the time course and sites of these changes. While most trophic phenomena appear to have a time course related to the length of remaining nerve stump, and estimates of the rate of axonal transport of this effect can be calculated, little is known for the case of muscle cholinesterase. An earlier study (Guth, et al, Exp. Neurol. 64: 236, 1964) reported that muscle cholinesterase decreased at the same time after denervation regardless of the length of the nerve stump. In order to more carefully examine the precise role of innervation in maintaining muscle cholinesterase we have begun by reexamining the possible relationship between nerve stump length and loss of muscle enzyme activity. Rat sciatic nerves were transected either at the level of branching into peroneal and tibial divisions (i.e., short stump) or at the emergence of spinal nerves L_4 and L_5 (i.e., long stump) on the left side. At intervals of 1 through 10 days, animals were sacrificed. Both innervated and denervated soleus and extensor digitorum longus muscles were removed and assayed for enzyme activity. The results show that during the first 24 hours no significant change occurs, but within 2 days a loss of 30% of the muscle enzyme activity is apparent. The enzyme activity continues to decrease until day 5, after which no further significant loss is observed. The data also show that the loss of enzyme is unrelated to nerve stump length and supports the earlier observations. Since the changes of enzyme activity are not directly correlated to the length of remaining nerve it must be suggested that this loss is not a "trophic" effect but reflects the loss of enzyme activity within the nerve terminals due to nerve transaction rather than muscle end-plate cholinesterase. Studies of cholinesterase activity in peripheral nerve using the same techniques are in progress, and preliminary results provide additional support for this proposal.

Muscle Cholinesterase Activity after Denervation

Days after Denervation	Long Stump EDL ± S.D.	Short Stump EDL ± S.D.	Long Stump Soleus ± S.D.	Short Stump Soleus ± S.D.
0	4.90 ± 1.00	4.90 ± 1.00	3.29 ± 0.79	3.29 ± 0.79
1	5.21 ± 1.17	4.67 ± 0.68	3.37 ± 0.65	3.64 ± 0.52
2	3.39 ± 0.29	———	2.38 ± 0.31	———
3	2.83 ± 0.57	3.43 ± 0.43	2.04 ± 0.21	2.24 ± 0.21
4	2.00 ± 0.72	3.55 ± 0.70	1.40 ± 0.15	1.99 ± 0.30
5	2.51 ± 0.43	3.12 ± 0.53	1.10 ± 0.20	1.27 ± 0.35
6	2.28 ± 0.40	2.46 ± 1.02	1.63 ± 0.42	1.15 ± 0.25
10	2.57 ± 1.14	1.46 ± 0.48	1.70 ± 0.73	0.98 ± 0.38

Data are expressed as uM ACh hydrolyzed/hr/muscle ± standard deviation.

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1498 NEURAL REGULATION OF CHOLESTEROL LEVELS IN MUSCLES OF GENETICALLY DYSTROPHIC CHICKENS. Michel P. Rathbone, Patricia A. Stewart and John D. Vickers*. Dept. Neurosciences, McMaster Univ., Hamilton, Ont. Canada L8S 4J9.

When neural tubes from chicken embryos with hereditary muscular dystrophy are transplanted into normal embryos they induce high thymidine kinase activity characteristic of dystrophic muscles in the muscles of normal hosts. Transplantation of normal neural tubes does not cause increased thymidine kinase activity (Rathbone et al. (1975) Science 189: 1106). In many muscular dystrophies the activity of membrane bound enzymes is abnormal (Roses et al. (1975) Nature 254: 350; Rodan et al. (1975) Nature 252: 589). These enzyme abnormalities may be due to changes in membrane fluidity observed in human myotonic dystrophy (Butterfield et al. (1974) Proc. Nat. Acad. Sci. (U.S.A.) 71: 909) and in chicken dystrophy (Sha'afi et al. (1975) Nature 254: 525). Because cholesterol has a major role in determining membrane fluidity we measured the cholesterol content of tissues from normal and dystrophic chickens at 9 days in ovo and subsequent stages of development. We also examined the influence of the genotype of the neural tube on cholesterol levels. At all ages examined the cholesterol content of the superficial pectoral muscles, that are affected by muscular dystrophy was significantly higher in dystrophic than in normal birds. For example at 11 days in ovo the cholesterol content of normal pectoral muscle was 2.87 $\pm$ 0.09 µg/mg muscle and of dystrophic pectoral muscle 3.40 $\pm$ 0.06 µg/mg muscle ($p < 0.001$). The ratio of cholesterol content in dystrophic and normal muscles was 1.185. In addition, the serum cholesterol was significantly higher in dystrophic embryos (2.86 $\pm$ 0.09 µg/µl serum) than in normal embryos (2.04 $\pm$ 0.10 µg/µl serum) ($p<0.001$), as was the liver cholesterol. However, the cholesterol content of brain tissue and of thigh muscles, which are unaffected by the dystrophic process, were not significantly different between dystrophic and normal animals.

Neural tubes from normal and dystrophic embryos were transplanted into normal hosts at 2 days in ovo. The cholesterol content of the pectoral muscles at 11 days in ovo was 2.63 $\pm$ 0.11 µg/mg. However, when neural tubes from dystrophic embryos were transplanted into normal hosts there was an increase in the cholesterol content in the pectoral muscles of the genetically normal host (3.15 $\pm$ 0.17 µg/mg). These results are significantly different from each other ($p<0.02$). Furthermore, the ratio of the cholesterol content in pectoral muscles of dystrophic neural tube——>normal host/normal neural tube——>normal host was 1.197, which is not significantly different from the dystrophic/normal ratio with unoperated birds (1.185). In contrast to the effects on the pectoral muscles the cholesterol content of other tissues, and of serum was not changed by the neural tube transplants. Transplants of normal neural tube into dystrophic host embryos resulted in a normal cholesterol content in the genetically dystrophic pectoral muscles. We conclude that the high cholesterol content of dystrophic pectoral muscles, which is a characteristic of muscular dystrophy is regulated by the neural tube. However, the raised cholesterol content of serum and liver in dystrophic chickens are pleiotropic effects of the dystrophic gene not directly related to the dystrophic changes in the muscle and are not regulated by the genotype of the neural tube.

1499 PREVENTION OF THE CHROMATOLYTIC RESPONSE IN RAT SUPERIOR CERVICAL GANGLION NEURONS BY NERVE GROWTH FACTOR. Norman R. West and Richard P. Bunge. Dept. Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

The observation that autonomic neurons of the superior cervical ganglion (SCG) retrogradely transport the protein nerve growth factor (NGF) raises the question of the functional significance of such a transport mechanism and the dependence of the neurons on this source of NGF (Hendry et al., Brain Res., 68:103, 1974). A recent study (Purves and Njå, Nature, 260:535, 1976) observed that exogenously applied NGF largely prevented the withdrawal of preganglionic synapses which is known to occur after crushing the postganglionic fibers. This suggests that NGF may function as a trophic factor transported from the periphery to the neuronal soma. The present study was undertaken to determine the morphological response of neurons to axotomy in the presence of exogenous NGF. The experimental model involved cutting the postganglionic axons as they course from the SCG to the head, and implanting a large dose of NGF in the region of the axotomy. Section of the nerve at this site is known to produce a classical chromatolytic response in many neurons of the SCG (Matthews and Raisman, Proc. R. Soc. Lond., 181:43, 1972). Adult female rats (180 to 250 gm) were anesthetized by intraperitoneal injection of chloral hydrate, and their right internal carotid nerves transected approximately 2 mm rostral to the SCG. Two 2 mm cubes of Gelfoam were implanted at the site of transection, each cube having been impregnated with 10 µl of phosphate buffered saline (PBS) containing (a) PBS alone, (b) 50 µg of 2.5S NGF, or (c) 50 µg of 2-ox-NGF (Frazier et al., Biochem., 12:3281, 1973). The surgical site was closed and the animals were allowed to recover, ptosis upon awakening confirming axotomy. At 2, 3 or 4 days after transection, the rats were again anesthetized and both SCG were removed, placed in buffered aldehyde fixative and quartered. The ganglia were subsequently postfixed with buffered OsO_4, dehydrated in acetone, embedded in Epon-Araldite and sectioned for light and electron microscopy. Ganglia treated with PBS or 2-ox-NGF contained many chromatolytic neurons. This response was characterized by displacement of the nucleus, contortion of the nuclear envelope, enlargement of nucleoli, accumulation of lysosomal appearing granules in the cytoplasm, and dispersion or peripheral displacement of Nissl bodies. On the other hand, neuronal somata from ganglia with cut axons which were treated with NGF did not differ significantly from control neurons in ganglia with intact nerves. These results suggest that the isolation of the neuronal soma from its peripheral source of NGF may be a dominant factor in the initiation of the chromatolytic response in autonomic neurons.

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1500 ELECTROPHORETIC CHARACTERIZATION OF NEURONAL BASIC PROTEINS IN SKELETAL MUSCLE. G. Appeltauer* and I.M. Korr. Kirksville College of Osteopathic Medicine and Michigan State University, College of Osteopathic Medicine.

In 1967 Korr, Wilkinson and Chornock (Science 155: 342) found that proteins synthesized in the perikarya of motor neurons are axonally transported and incorporated into the muscle cells they innervate. Further studies have led to the electrophoretic characterization of some nerve-to-muscle delivered acid proteins that are axonally transported to skeletal muscles.

3H-lysine was administered to the 4th ventricle of rabbits. The animals were sacrificed 1-70 days following the administration of the precursor. Soluble extracts from the hypoglossal nerve and styloglossus muscle were fractionated by disc-electrophoresis at pH4.2 and 15% gel concentration. In the hypoglossal nerve gels, total radioactivity was highest at day 12 after the 3H-lysine administration. It was concentrated mainly at the anodic end and in one stained band. Five other labelled proteins also appeared in the gels. The nerve-delivered radioactivity in the styloglossus muscle gels was highest at day 34. It was highly concentrated at the anodic end of the gels and in one protein band, and less concentrated in 4 other proteins. The electrophoretic mobilities of the radioactive proteins in the nerve gels and of equivalent radioactive proteins in the muscle gels were the same. Nerve basic proteins seem to travel without obstacle along the axons to the nerve terminals, and from there they slowly penetrate the muscle. There is no change in the electrophoretic mobility of the proteins during this process.

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1501 EFFECT OF ELECTRICAL STIMULATION ON METABOLISM OF EXTRAJUNCTIONAL ACETYLCHOLINE RECEPTORS IN DENERVATED MAMMALIAN MUSCLE. Diana Card* and Douglas M. Fambrough. Dept. of Embryology, Carnegie Institution of Washington, 115 W. University Parkway, Balto, Md. 21210.

After supersensitivity to acetylcholine has developed in denervated mammalian skeletal muscle, electrical stimulation of the muscle can lead to a decline in extrajunctional acetylcholine (ACh) sensitivity nearly to the level measured in innervated muscles. These changes in ACh sensitivity reflect changes in numbers of extrajunctional ACh receptors, and these changes should be understandable in terms of the incorporation of receptors into the membrane and their subsequent degradation.

The degradation rate of extrajunctional ACh receptors can be inferred from measurements of the rate at which ^{125}I-labeled α-bungarotoxin bound to these receptors is degraded with concomittant release of radiolabeled degradation products from the muscle fibers. This degradation rate is approximately 2% per hour in unstimulated, denervated mouse extensor digitorum longus muscle in perfusion culture, and thus the half-life of ACh receptors in the plasma membrane averages about 25 hours under these conditions.

Degradation rate does not appear to change during the first two weeks of denervation. The same pattern of electrical stimulation which has been shown *in vivo* to promote rapid decline in extrajunctional chemosensitivity results in a reduction in degradation rate measured *in vitro*. The reduction is independent of length of the denervation period. Since chemosensitivity declines during electrical stimulation without any acceleration of degradation, it follows that electrical stimulation must repress the biosynthesis or incorporation of new receptors into plasma membranes. We are testing this hypothesis and will report about it in November.

1502 BENEFICIAL EFFECTS OF DIPHENYLHYDANTOIN ON HEREDITARY MUSCULAR DYSTROPHY OF THE CHICKEN. Richard K. Entrikin, Paul M. Weidoff*, Gary T. Patterson*, Karen L. Swanson*, and Barry W. Wilson. Depts. of Avian Sciences and Pharmacology, Univ. California, Davis, CA. 95616.

A chick affected with hereditary muscular dystrophy cannot right itself when placed on its back. Electrophysiological abnormalities of the skeletal muscle fiber membrane suggest that myotonia may be a factor in the righting difficulty. We injected dystrophic chicks, i.p., with 20 mg/Kg diphenylhydantoin (DPH), an antimyotonia drug, at 12-hour intervals on days 1-40 ex ovo. Righting ability was tested twice-daily by placing each chick on its back until it could no longer right itself. Since the testing procedure was a form of exercise, which alone might have affected righting ability, we also tested uninjected dystrophic chicks. Exhaustion scores (consecutive number of times a chick could right itself in a single test period) were obtained for each chick just before the second daily injection. Mean exhaustion scores for days 13-35 were: (1) untreated normal, 19.5; (2) untreated dystrophic, 1.8; (3) exercised dystrophic, 8.9; and (4) DPH-treated-exercised dystrophic, 20.3 . We also determined spectrophotometrically the activities of lactic dehydrogenase (LDH) and acetylcholinesterase (AChE) in posterior latissimus dorsi (PLD) muscles. LDH activities (ΔOD/g/min) were: (1) untreated normal, 1909; (2) untreated dystrophic, 1023; (3) exercised dystrophic, 2150; and (4) DPH-treated-exercised dystrophic, 1643. AChE activities (ΔOD/g/min) were: (1) untreated normal, 1.37; (2) untreated dystrophic, 5.95; (3) exercised dystrophic, 5.45; and (4) DPH-treated-exercised dystrophic, 1.40. It appears that the combination of DPH and moderate exercise is more beneficial than exercise alone, and that DPH may correct some abnormality that is intimately associated with AChE regulation. (Supported by USPHS, NIH grants NS 05308 and NS 10957 and the MDA)

1503 STUDIES OF A NERVE EXTRACT WITH TROPHIC PROPERTIES. B. W. Festoff and S. Israel*. NINCDS, NIH, Bethesda, MD. 20014.

A protein extract of rat sciatic nerve axoplasm (SNP) has been partially purified and characterized. When added to dissociated primary cultures of chick pectoral muscle, in vitro, a marked "trophic" response occurred. An increase in number and diameter of myotubes was found when SNP-treated cultures were compared with control (albumin-treated) cultures. A marker for excitable membrane function in these cultures, binding of $^{125}I\alpha$ bungarotoxin (α BT) to the cholinergic receptor, was also influenced by SNP. An increase in the specific number, more rapid appearance, and much more rapid disappearance of α BT binding occurred in SNP-treated cultures.

On SDS gels the major protein in SNP was a 67,000 daltons polypeptide. High specific activity choline acetyltransferase (CAT) was present in the SNP (3500 pmoles x min $^{-1}$ x mg protein $^{-1}$). Sheep immunized with SNP produced several IgG antibodies to proteins in the SNP and developed an unusual "allergic" neuropathy with features of terminal axon dysfunction and neurotransmission failure (Festoff, Israel & Engel Neurology in press).

In vitro inhibition of CAT activity by anti-SNP IgG was found. With increasing amount of IgG protein from the most severely involved sheep, CAT activity in SNP was reduced by 65%. Preliminary studies on the effect of anti-SNP on SNP trophic effects in muscle cultures suggest that an "anti-trophic factor" had been produced. Preincubation of SNP and anti-SNP (4°C) tended to abolish the effects of SNP on the muscle cultures. These initial studies support the notion that factors "trophic" for muscle exist in the axoplasmic compartment of nerve. The in vitro system offers advantages for testing this hypothesis further, and to clarify the role, if any, CAT has in the "trophic" effect.

1504 SURVIVAL OF NEURONS IN XENOGRAFTS OF SENSORY GANGLIA IN THE NUDE MOUSE. Harry G. Goshgarian*, George C. Creswell*, and Andrew A. Zalewski. (Spon: Herbert Yellin). LNC, NIH, Bethesda, Md. 20014.

Mice homozygous for the recessive gene mutation nude are hairless and suffer from aplasia of the thymus. One consequence of thymic aplasia is deprivation of the cell mediated immunity associated with T-lymphocytes, a deficiency demonstrable by the nude's inability to immunologically reject allografts or xenografts (grafts between different species). The present study was performed to determine whether neurons in xenografts would survive in the nude mouse so that subsequently we could determine whether the trophic influence of sensory neurons on taste buds (i.e., the neural induction and maintenance of buds) might be mediated between tissues of different species. Rat nodose ganglia were transplanted to the anterior chamber of the eyes of normal NIH-mice or to nude mice. All rat ganglia were immunologically rejected at 3 weeks in normal mice; these rejected ganglia lacked neurons and were infiltrated by neutrophilic leukocytes and mononuclear cells. On the other hand, nude mice accepted rat sensory ganglia at 3 weeks with the result that many neurons survived and no cellular infiltration was present. Preliminary findings also indicate that the surviving rat neurons in the nude mouse are functional since rat neurons regenerated nerve fibers into cotransplanted mouse tongue grafts and induced taste buds. These results demonstrate that xenogenic neurons can survive and function in the nude mouse. The eye of the nude mouse, therefore, may be an ideal *in vivo* culture system to study xenogenic tissue reactions which are not readily performed in tissue culture.

1505 EFFECT OF DENERVATION OF RNA POLYMERASE ACTIVITIES IN NUCLEI ISOLATED FROM SLOW AND FAST SKELETAL MUSCLES. I.R. Held, R.T. Rodrigo* and H.C. Yeoh*. Neurosci. Res. Lab., VA Hosp., Hines, Il. 60141 and Loyola Univ. Med. Ctr., Maywood, Il. 60153.

The optimum incubation conditions for assaying RNA polymerase I and II in nuclei isolated from the soleus and extensor digitorum longus (EDL) muscles of the rat were evaluated by systematically varying 1) the Mg^{2+} or Mn^{2+} concentration at low and high ionic strength; 2) the ionic strength with $(NH_4)_2SO_4$; 3) the concentration of nucleotide triphosphates; and 4) the number of muscle nuclei incubated. The DNA-dependency of the reaction was demonstrated by the *in vitro* inhibitory effect of deoxyribonuclease and Actinomycin D. The specific activity of the Mn^{2+} + $(NH_4)_2SO_4$-activated enzyme (pmoles of ^{14}C-UTP incorporated into acid-insoluble material/time/mg DNA) in nuclei from both solei and EDL was about fivefold greater than the Mg^{2+}-activated enzyme of each muscle.

The RNA polymerase activities were also measured in nuclei isolated from solei and EDL muscles which had been denervated for various periods (i.e., 3,25,49,72 and 120 hours) by cutting the sciatic nerve 40-45 mm from its insertion into these muscles and simultaneously in nuclei from the sham-operated, contralateral muscles. Between 25-72 hours the specific activities of both the Mn^{2+} + $(NH_4)_2SO_4$ and the Mg^{2+}-activated enzymes were 130 to 150% higher in nuclei from denervated muscles (either solei or EDL) than in sham-operated, muscle nuclei. Enzyme activity was not significantly elevated in the 120 hour denervated muscle. Our results show that biochemical alterations occur in muscle nuclei after denervation, but whether these are attributable to the influence exerted by the motor neuron through cholinergic transmission, other neurotrophic factors or both of these is still not clear. Supported by NINDS grant NS-11755 from USPHS.

1506 SPECIFIC CONDUIT GUIDANCE AFTER NERVE CRUSH AND ITS ABSENCE AFTER PERIPHERAL NERVE TRANSECTION IN THE CAT. K. W. Horch. Dept. Physiol., Coll. Med., Univ. Utah, Salt Lake City, UT. 84132.

The innervation pattern of type I cutaneous mechanoreceptors was mapped before and 3 months after crush of the femoral cutaneous nerve. The regenerated type I neurons duplicated the original innervation pattern, implying that the regrowing sprouts followed their old endoneurial tubes back to the skin. Replication of the innervation pattern was not seen after recovery from nerve transection. This provides the first direct experimental support for the concept that successful neuronal regeneration after nerve crush is due to the guidance of the fibers back to appropriate terminal locations by their old Schwann tubes. Recovery after transection is less complete because the regenerating fibers lack this guidance.

1507 EFFECT OF DENERVATION ON ACETYLCHOLINESTERASE, BUTYRYLCHOLINESTERASE AND POLYAMINES IN SKELETAL MUSCLE. Leon T. Kremzner*, Virginia M. Tennyson, and Armand F. Miranda, Coll. of P & S, Columbia Univ., N.Y., N.Y. 10032

Denervation of skeletal muscle results in a profound species dependent alteration of muscle biochemistry. In the rat, cholinesterase activity progressively declines (Guth et al., 1964); however, in the mouse and rabbit, studied here, it does not. The polyamines, which modulate RNA and protein synthesis, were also studied. The "soluble" and particulate fractions of muscle homogenates were assayed by using (^{14}C) acetyl-β-methylcholine for acetylcholinesterase (AChE), and (^{14}C) butyrylcholine and an AChE inhibitor, for butyrylcholinesterase (BuChE). Polyamines were determined on a PCA extract of whole muscle with an amino acid analyzer. In both the mouse and rabbit, muscle denervation results in a progressive increase in putrescine and spermidine, the increase being as large as 10 fold; spermine was not appreciably altered. In mouse, AChE activity was essentially unaltered at 1.5, 3 and 5 weeks following nerve section; BuChE activity was elevated in the soluble, but not the particulate fraction at 3 and 5 weeks. In the rabbit, AChE activity, beginning at about 2 weeks, is progressively increased in both the "soluble" and particulate fractions; by the 6th week there is a 15 fold and 5 fold increase respectively. "Soluble" and particulate BuChE is also progressively elevated by a factor of 5. Our results indicate that the pattern of AChE and BuChE synthesis depends on the species. Alterations in polyamine metabolism do not appear to be species dependent. (Supported by Muscular Dystrophy Association of America and NS 11766).

1508

WITHDRAWN BY AUTHOR

1509 ROLE OF MULTIPLE INNERVATION ON THE EXPRESSION OF DYSTROPHY IN AVIAN MUSCLES. Jacob Mazliah *, Jane Butler* and Ethel Cosmos. Dept. Neurosciences, McMaster Univ., Hamilton, Ontario, Canada L8S 4J9.

In chickens with hereditary muscular dystrophy the fast twitch posterior latissimus dorsi (PLD) characterized by a focal innervation and by anaerobic metabolism shows an early progression of the disease; in contrast, the slow tonic anterior latissimus dorsi (ALD) characterized by a multiple innervation and by aerobic metabolism lacks phenotypic signs of dystrophy. Our comparative analysis of the ontogeny of these two muscles in normal birds reveals that whereas the slow ALD has established its adult chemical profile by the end of the embryonic period, the fast PLD demonstrates an alteration of its metabolic activity from aerobic (embryonic) to anaerobic during ex ovo ontogeny. A similar switch in metabolic activity is incomplete in fast twitch muscles of dystrophic genotype with the result that dystrophy is expressed in these muscles as an inability to complete differentiation. Since both muscle types are genotypically dystrophic then we might assume that the phenotypic characteristics of the disease are expressed only when the muscle is challenged to alter its metabolic behaviour. The ALD in which such metabolic demands are suppressed, presumably by its specific multiple innervation, does not demonstrate dystrophic symptoms. Our strategy is to remove the demand for metabolic alteration from the PLD by supplying it with the nerve normally innervating the ALD. The "slow" nerve from the excised ALD is placed on the distal end of the denervated PLD; further, the PLD is injured in situ to insure rapid innervation by the foreign nerve. Both histochemical and physiological evidence will be presented to test the hypothesis that the slow tonic muscles of the bird are spared of dystrophic symptoms due to their normal "arrest in differentiation". Supported by a special grant from the Muscular Dystrophy Association, Inc.

1510 NEURAL REGULATION OF THE ACTIVITY OF CERTAIN OXIDATIVE ENZYMES OF TYPE I MUSCLE. Patti M. Nemeth* and R. A. Pieter Kark. Dept. Neurology, Sch. Med., UCLA, Los Angeles, CA 90024.

To test whether oxidative metabolism of Type I muscle is controlled by nerve, soleus muscles of guinea pig were either denervated or rendered immobile by skeletal fixation. Two to eight weeks later, whole muscle weights, histochemistry of transverse sections, and specific activities of enzymes in muscle homogenates were measured. The denervated soleus was compared to the contralateral sham-operated control; the immobilized soleus was compared to the contralateral unoperated control. All fibers of operated muscles were atrophic by microscopy. Weights of denervated muscle fell to 53.7 ± 3.2% of controls (mean ± SEM), and of immobilized, to 55.6 ± 3.9%, averaged over the two to eight week period.

Denervation led to a progressive fall in enzyme activities. Thus, at 4 weeks, succinate dehydrogenase fell to 50.2 ± 4.2%, cytochrome-c oxidase to 56.8 ± 4.9%, and lipoamide dehydrogenase to 58.8 ± 7.3% of control. On the other hand, those activities remained constant or increased in immobilized muscle; they were 102.0 ± 4.7%, 108 5 ± 4.9%, and 113.3 ± 5.3% of control respectively. The changes in denervated and immobilized muscle differ with $p < 0.0005$. These findings suggest a trophic neural control of these mitochondrial enzymes of muscle, a control which is independent of muscle activity. (Supported by a grant from the Muscular Dystrophy Association)

1511 SPECIFIC INNERVATION OF SYMPATHETIC GANGLION CELLS. A. Njå* and D.Purves, (SPON: A.L. Prensky), Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

The innervation of guinea pig superior cervical ganglion cells by nerve fibers emerging from the first seven thoracic spinal segments was studied by means of intracellular recording while stimulating the ventral roots in vitro. Each neuron receives innervation from multiple thoracic segments (mean 4, range: 2-7) and is contacted by more than 10 preganglionic axons, on average. Individual neurons were almost always innervated by contiguous spinal segments. Thus the pattern of innervation was different from that expected if preganglionic fibers established synaptic connections in a random fashion. Neurons tended to be most strongly innervated by axons from one spinal segment, with adjacent segments generally contributing a synaptic influence that diminished as a function of their distance from the dominant segment. Although most neurons were preferentially innervated by T_2 or T_3, any of the first six thoracic segments could provide the dominant innervation of particular nerve cells. This synaptic organization is presumably the basis for the finding that stimulation of individual thoracic ventral roots in vivo produces different but overlapping patterns of sympathetic effects. The way in which ganglion cells express a graded preference for a particular set of preganglionic fibers is as yet unknown.

1512 DEVELOPMENTAL INTERACTION OF DYSTROPHIC AND NORMAL CELLS IN THE NEUROMUSCULAR SYSTEM OF MOUSE CHIMERAS. A.C. Peterson. MRC Group in Developmental Neurobiology, Dept. Neurosciences, McMaster Univ., Hamilton, Canada.

Mice with hereditary muscular dystrophy (gene symbol dy) present with two very striking phenotypic abnormalities - muscle wasting and amyelination of spinal and cranial nerve roots, both motor and sensory. Although numerous experiments designed to establish the primary site of action of the dy gene in relation to the muscle pathology have been done, including muscle transplantation, parabiosis and in vitro studies, the nature of the gene lesion and its primary site of action remains unknown. The relationship of the two abnormalities: one a progressive degeneration of muscle, and the other a developmental block in normal axon-Schwann cell relationships, is also enigmatic. We have produced mouse chimeras by aggregation of normal and dystrophic preimplantation embryos. Both the normal and dystrophic cells differentiate within a single animal; theoretically there is an ideal opportunity to investigate the developmental interaction of muscle, nerve and various extraneuromuscular systems, in the expression of the abnormal phenotypes. The genetic constitution of the chimeras produced include: SWV +/+<—>C57BL/6Jdy^{2J}/dy^{2J}; 129 +/+<—>C57BL/6Jdy^{2J}/dy^{2J} and 129dy^{1J}/dy^{1J}<—>C57BL/6J +/+. These chimeras (examined up to 1½ years old) have not presented any clinical features of dystrophy. However, the constitutional genotype of individual muscles, determined by electrophoretic analysis of strain specific isoenzymes, varies from predominantly normal to predominantly dystrophic. Similarly, the spinal roots demonstrate a broad range of axon genotypes. Preliminary histological studies indicate that the presence of dystrophic myonuclei do not result in muscle pathology. Results bearing on the question "Are the axons, or the Schwann cells, or both, responsible for the abnormal myelination in the dystrophic mouse?" will also be reported.

1513 COMPETITIVE AND NON-COMPETITIVE RE-INNERVATION OF MAMMALIAN NEURONS. D.Purves, Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

The ability of native and foreign (vagal) nerve fibers to re-innervate neurons of the guinea pig superior cervical ganglion, either alone or in competition with each other, was studied by means of intracellular recording and electron microscopy. Native (sympathetic preganglionic) fibers make synaptic contacts with nearly all ganglion cells within one month of cervical trunk section; within 6 months the degree of innervation, judged by measurement of excitatory postsynaptic potential (e.p.s.p.) amplitude and electron microscopical synapse counts, approaches normal. However, even after 15 months innervation was weaker than in normal ganglia. A similar number of vagal fibers grow into denervated ganglia and make contact with nearly all ganglion cells within one month, yet after 6-12 months e.p.s.p. amplitudes in response to foreign nerve stimulation remain relatively small, and counts of synapses are only about half as great as in normal ganglia. When both native and foreign fibers are allowed to re-innervate ganglion cells simultaneously, about half the neurons in the ganglion receive synapses from both sources after one month. The proportion of dually innervated cells remains roughly constant for at least 15 months. Thus although these neurons are generally re-innervated more effectively by native than foreign fibers, foreign synapses are stable and show no tendency to be replaced by native terminals.

1514 EARLY CHANGES IN MUSCLE GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY AFTER DENERVATION: HISTOCHEMICAL LOCALIZATION AND DEPENDENCE ON NERVE STUMP LENGTH. Norman Robbins and Deborah Hall*. Dept. Anat., Sch. Med., Case Western Res. U., Cleveland, Ohio 44106.

Glucose-6-phosphate dehydrogenase (G6PD) activity is elevated in developing, denervated and regenerating muscle. We found that G6PD activity of rat diaphragm is increased 30±5% ($n=22$) over paired control 12 hrs after cutting the phrenic nerve close to the muscle. In contrast, 12 hrs after a subclavicular denervation (leaving a 3 cm nerve stump attached to the muscle), G6PD activity decreased 11±3% ($n=7$). Both changes were significantly different from 0% and from eachother. At 24 and 48 hrs, G6PD activity was elevated 59% and 103%, respectively, with no difference at 24 hrs between proximal and distal denervation. Histochemical studies showed increased reaction product in both muscle fibers and unidentified interstitial cells of 12 hr distally denervated muscle. We suggest that a rapidly acting neurotrophic mechanism, independent of usage or muscle stretch, regulates a key enzyme in muscle metabolism.

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1515 CELL DEATH IN THE DEVELOPING TROCHLEAR NUCLEUS. G.S. Sohal. Department of Anatomy, Medical College of Georgia, Augusta, Georgia. 30902.

The development of the trochlear nucleus under normal conditions and in the absence of its peripheral field of innervation was studied, from day 7 of incubation age through hatching, in the White Peking duck embryos. The optic primordium and the surrounding mesoderm was removed, usually on the right side, on the fouth day of incubation. Data on cell number and size on day 12, when the nucleus attains maximum number of cells, indicate no significant differences between the control and the affected nucleus. Removal of the superior oblique muscle thus has no effect on proliferation and differentiation of the cells till day 12. There is, however, a 50% cell death in the control nucleus between days 13 and 21. An additional hypoplasia in the affected nucleus is observed from days 13 to hatching. Magnitude of cell death in the affected nucleus varies from 85% death to a virtual absence of the cells at hatching. Two morphologically distinct cell types, e.g., large well-differentiated, and small relatively undifferentiated cells are present in the control and the affected trochlear nucleus during the early period of morphogenesis. As the development proceeds there is a progressive decrease in the relative number of the small cells. It is possible that the large cells may differentiate early and their neurites establish connections with the superior oblique muscle; small cells on the other hand may be slow in development and do not contribute axons to the trochlear nerve. It can be hypothesized that during normal development the survival of the large cells may be due to the establishment of their appropriate peripheral connections while the death of the small cells is related to their failure to send out axons to the periphery. The additional hypoplasia in the affected nucleus might be due to the failure of the growing axons to make appropriate synaptic contacts with the periphery.

1516 NERVE STUMP LENGTH DEPENDENCE OF SENSITIVITY TO CHOLINERGIC DRUGS IN FROG AND RAT DENERVATED MUSCLES. Osvaldo D. Uchitel and Norman Robbins. (SPON: M. Singer). Dept. Anat., Sch. Med., CWRU, Cleveland, Ohio 44106.

The contractile response to cholinergic agents have been studied in normal and denervated muscles of the frog and rat.

Innervated gastrocnemius slow muscle of the frog developed a 50% of its maximum response (ED_{50}) when 2×10^{-5} M carbachol was applied. Three days after short stump nerve denervation (0.5 cm.), the sensitivity to carbachol started to increase and reached a maximum after 14 days (ED_{50} 1.95×10^{-6}M). The same sequence, but with three days delay, was seen following long stump denervation (4.6 cm.).

The isotonic response of an innervated rat diaphragm to 10 ug/ml. of acetylcholine was 1% of the response to 0.1 M K_2SO_4. About 30 hr after cutting the phrenic nerve close to the muscle (0.5 cm.), the response increased to 55%. The response of a long stump denervated muscle was 5% at this time and did not develop significantly until about four hours later. When the long nerve stump was removed 8 hr after the initial denervation, the increase in acetylcholine sensitivity was still delayed.

These experiments suggest that in both frog and rat, there is neurotrophic regulation of the sensitivity to cholinergic agents. Although the presence of a long nerve stump delays the appearance of increased acetylcholine sensitivity from 30 to 34 hr after denervation, the action of the nerve takes place within the first 8 hr after denervation.

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1517 THE PERMANENT SURVIVAL AND FUNCTION OF NEURONS IN AG-B HISTOINCOMPATIBLE ALLOGRAFTS OF GANGLIA IN IMMUNOLOGICALLY TOLERANT RATS. Andrew A. Zalewski and Willys K. Silvers*. LNC, NIH, Bethesda, Md. 20014 and Dept. of Human Genetics, Univ. of Pa. Sch. Med., Philadelphia, Pa. 19174.

Previous studies demonstrated that neurons in Ag-B histoincompatible allografts of sensory ganglia are rejected unless the recipients have been rendered immunologically tolerant. The present study was performed to determine how long Ag-B incompatible neurons would survive and function (i.e., regenerate their nerve fibers and form functional connections) in tolerant recipients. Sensory ganglia(vagal nodose) were taken from neonatal Lewis (LE) rats and one LE ganglion transplanted to the anterior chamber of each eye and each sternomastoid muscle of normal adult Brown Norway (BN) rats or to adult BN rats previously made tolerant to LE antigens. Normal BN rats rejected all four neonatal LE ganglia (i.e., no neurons were present) by 70 days, but tolerant BN rats accepted neonatal LE ganglia (i.e., neurons were present) even after 429 days. Furthermore, when isografts of tongue were placed over LE ganglia that had been in the eyes of tolerant BN recipients for 429 days, nerve fibers from surviving LE neurons grew into the tongue grafts and caused the regeneration of taste buds. Tongue grafts transplanted to eyes that did not have ganglia failed to develop buds. These results show that the immunological rejection of neurons is permanently prevented in tolerant animals and that long-term surviving neurons in tolerant hosts are functional in that they retain the ability to regenerate their nerve fibers and form functional connections (i.e., induce taste bud formation).

Vestibular System

1518 QUANTITATIVE ANALYSIS OF NERVE FIBER DISTRIBUTION WITHIN THE HORIZONTAL AMPULLARY NERVE OF THE GUITARFISH SEMICIRCULAR CANAL. Robert F. Dunn, Dennis P. O'Leary, Dept. Surg. UCLA Sch. Med., Los Angeles, Ca., and Dept. Otolaryngol., Univ. Pittsburgh, Pittsburgh, Pa.

Dynamic response characteristics obtained from horizontal ampullary nerve (HAN) fibers were shown previously to vary according to the position of the fibers within the HAN which is comprised of five discrete bundles (1). Nerve fibers located within the central HAN bundle were both more sensitive and displayed faster response dynamics than fibers located in the anterior or posterior bundles. We tested whether the nerve fiber diameter distribution also varied as a function of their location within the HAN. A computerized analysis system was developed to digitize dimensional and positional information directly from photomicrographs (2). Nerve fibers from over 15 HAN preparations were digitized and stored as computer files for subsequent statistical analyses. Nerve fiber diameter distributions from the digitized files of each nerve bundle were characterized in the form of means, standard deviations, coefficient of variation and histograms for both axon and axon-plus-myelin diameters. The nonparametric Kruskal-Wallis analysis of variance test (3) was used to determine whether the fiber sizes were distributed differently among the five bundles of the HAN. The largest fibers were clustered in the central bundle 3, whereas the smallest fibers were located in the extreme anterior and posterior bundles, 1 and 5, with an intermediate size distribution in bundles 2 and 4. These results were significant at a $p<0.01$ within each HAN. Moreover, pair-wise multiple comparisons (4) among the five bundles revealed fiber size distribution differences between the bundle pairs 1,3 and 3,5 in all individual preparations at a level of $p<0.01$. Similar analyses on specific areas within each bundle, for example center versus periphery, also demonstrated preferential size distributions of the nerve fibers. These results will be discussed in terms of the nerve fiber projections to the horizontal crista, and to the response characteristics of the individual nerve fibers.

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1519 CROSS-CORRELATION ANALYSIS OF CAT VESTIBULO-OCULAR DYNAMICS USING VESTIBULAR WHITE NOISE INPUTS. Dennis P. O'Leary, F. Owen Black and Conrad Wall, III Dept. of Otolaryngology, Eye and Ear Hospital, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15213.

Analysis of the vestibulo-ocular motor system's response to sinusoidal or constant angular acceleration is complicated by the fact that only portions of the saccadic waveforms (e.g. the slow phase) can be related to the rotational stimulus. Alternatively, a waveform analysis based upon cross-correlation of the system response to a pseudorandom angular acceleration input automatically selects only the components that are correlated with the input and rejects all other waveform components regardless of their source. Moreover, bandlimited white noise can be considered the equivalent of superimposed sine waves of all frequencies spanning a selectable bandwidth and is therefore an efficient stimulus.

Silver wire electrodes, implanted in the orbital rim, were used for chronic recording of electro-oculographic (EOG) potentials generated during eye movements. The EOG was calibrated in units of absolute eye position by pre-test scanning with a computer-controlled image dissector camera programmed to track the center of the pupil. Awake, unanesthetized cats, trained to be head restrained in a box, were rotated in a horizontal plane using a servocontrolled rotating table interfaced with a PDP-11 computer system. The vestibular stimulus was a pseudorandom binary sequence (PRBS) of rotational acceleration, having an effective band width of 0.01 - 10 Hz. Eye movement EOGs, filtered for a pass band from 0.01 to 100 Hz, were digitized and cross-correlated on-line with the PRBS input. The resulting cross-correlation was corrected and scaled to result in a linear system unit impulse response (UIR), or time domain equivalent linear filter characteristic, for the vestibulo-ocular system.

The resulting impulse responses displayed an initial time delay followed by a rise to a maximum amplitude and a slower decay toward the baseline, with varying degrees of baseline undershoot. Moreover, the impulse response amplitudes decreased and showed systematic changes in response profile as the input acceleration magnitude increased. Rhythmic saccades resembling nystagmus occurred in the EOG responses only when the table velocity exceeded a threshold which varied from animal-to-animal. On the basis of these preliminary results, we conclude that cross-correlation with white noise rotational inputs is an efficient technique for on-line determination of the linear response characteristics of the vestibulo-ocular system, by the automatic exclusion of both uncorrelated noise sources and information from other converging sensory systems.

1520 AN UPPER LIMIT ON THE PHYSIOLOGICAL RANGE OF CUPULA MOTION IN THE SEMICIRCULAR CANAL OF THE SKATE . Charles M. Oman*, Lawrence S.Frishkopf, and Moise H. Goldstein. Massachusetts Institute of Technology,Cambridge, MA.02139, The Marine Biological Laboratory, Woods Hole, MA.02543,and Johns Hopkins University, Baltimore, MD. 21218

There has been a recent research interest in the histology of the attachment and the magnitude and mode of motion of the semicircular canal cupula. Theoretical considerations (C.M. Oman and L.R. Young, Acta Oto-Laryng.74;324-331,1972) predict that the dynamic range of motion of the cupula midpoint in the human semicircular canal is from about 10^{-2} μm at threshold up to about 3 μm at the upper limit of self induced sinusoidal head motion. It has often been tacitly assumed that much larger motions of the cupula within the ampulla typify the physiologic behavior of this structure. We attempted to determine experimentally the upper bound of cupula motion in an isolated preparation of the labyrinth of the skate Raja erinacea, where theoretical considerations suggest that cupula motions should be, if anything, larger than those of the human cupula when subjected to the same angular accelerations. The animals were pithed and the ears excised and kept immersed throughout the entire experiment in a Ringers solution chosen to match skate perilymph. The horizontal and anterior ampullary nerves were exposed, and single and multi-unit activity monitored on nerve twigs raised from the bath on a wire hook electrode. In initial experiments in which the canal was cut, cannulated, and the cupula hydraulically driven, cupula midpoint displacements of 30-50 μm were required to produce increments in evoked responses equal to spontaneous levels of activity. The cupula was seen to deflect as a swinging door. However, we became concerned about the possible traumatic effects on the preparation of the cannulation procedure. To avoid this problem, we switched to caloric stimulation of the canal. We found that the small endolymph density differences produced by heating the closed canal with a focussed spot of light stimulated afferent units in the ampullary nerve in a repeatable way. By moving the light spot, excitatory or inhibitory responses could easily be produced. As expected, no response was observed when the canal was in a horizontal plane. Some afferent units were found to be tonic in their response characteristics to a constant illumination stimulus, while others appeared phasic. Phasic units often appeared to have higher thresholds. The small amount of adaptation seen in some units strongly suggested that cupula displacement as a result of sustained caloric stimulation was statically maintained. To visualize the cupula, a small puncture was made through the ampullary wall with a sharpened pipette filled with Alcian blue dye (0.5-1.0%) in simulated skate endolymph. The dye solution was gently infused to stain local regions of the cupula. It was established that puncture and injection usually had no significant effect on spontaneous activity or evoked response by comparing responses to a standard caloric stimulus before and after these procedures. Cupula motion was monitored visually by observing stained regions through the ampullary wall with a calibrated eyepiece reticle in a dissecting microscope. Caloric stimuli which doubled the level of afferent activity in individual units produced no detectable motion of any region of the cupula examined in these preparations. However, motion of the top of the cupula was observed in response to caloric stimuli in preparations in which the cupula was first visibly displaced by rapid injection of dye. Because of the large change in frequency of the afferent response produced with the caloric method, we conclude that the physiologic range of cupula motion in this animal is less than the resolution of our method, which we conservatively estimate as 3-5 μm. Experimentally observed motions an order of magnitude larger are, in our view, unphysiological, and result from traumatization of the cupula.

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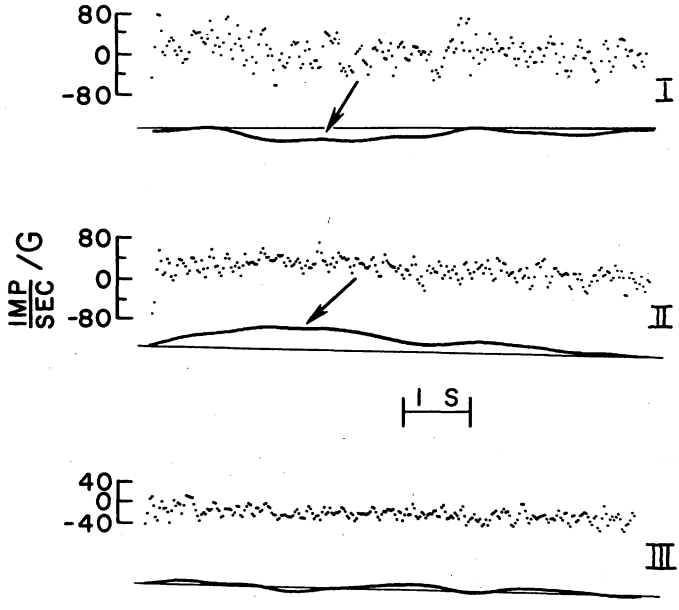
1521 ALTERATION OF GUITARFISH UTRICULAR DYNAMICS BY CHANGING ORIENTATION IN THE GRAVITATIONAL FIELD. Conrad Wall, III and Dennis P. O'Leary. Dept. of Otolaryngology, Eye and Ear Hospital, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213.

Responses of first order utricular afferents are usually classified as some combination of tonic, referring to a static orientation with respect to the gravity vector, versus phasic, referring to a dynamic response to a changing acceleration. We describe a class of cells having dynamic properties which can be altered by the orientation of the polarization vector in the gravity field. The cell's dynamic properties were estimated from the unit impulse response (UIR) determined by cross-correlation of the neural response to a white noise tangential acceleration applied parallel to the polarization vector. The centripetal acceleration was less than 10% of tangential. The influence of the gravitational effect was varied independently by tilting the table plane from horizontal to vertical and by precessional effects which slowly rotated the head in the gravity field during successive periods of acceleration stimulus.

These cells have three response components: (1) a "tonic" steady-state firing rate which changes with head orientation in the gravity field; (2) a "fast phasic" underdamped portion of the UIR which is independent of head position; (3) a "slow phasic" part of the UIR that depends upon head orientation. Figure 1 shows unfiltered (upper) and filtered (lower) impulse responses indicating how the two phasic components are influenced in three head orientations: I. Normal, II. Upside down, III. Head on side. The left hand side of the unfiltered UIRs show the "fast phasic" response. This component is the same for normal (I) and upside down (II), but is reduced in magnitude in the side orientation (III). The low-pass filtered UIRs show the change in the "slow phasic" component from concave upwards (I) to convex upward (II) (see arrows). This component is not detectable during table rotation in the horizontal plane (III). This "slow phasic" component of the UIR suggests that cell dynamic response to sudden accelerations are altered in a systematic way by the orientation of the polarization vector with respect to gravity. (Supported by NIH grants NS 05206-01 and NS 12494-02.)



1522 PROPERTIES OF VESTIBULAR NEURONS PROJECTING TO THE NECK SEGMENTS OF THE CAT SPINAL CORD. V.J. Wilson, Y. Uchino*, A.J. Susswein* and S. Rapoport*. Rockefeller University, New York, N.Y. 10021.

Because the vestibular system has a special relationship with the neck, we have studied further the properties of vestibular nucleus neurons projecting to the upper cervical segments.

Neurons projecting to the neck were identified by antidromic microstimulation in the C_3 gray matter of anesthetized or decerebrate cats. Thresholds were never over 25 μA and were usually 15 μA or less; measurements of threshold and distance to the nearest vestibulospinal tract showed that no axons were activated by current spread to the tracts. Recording in the vestibular nuclei was extracellular and most recording locations were marked with Fast Green dye. As expected neurons in the ipsilateral descending, medial and lateral nuclei projected to C_3; 70% of our sample was in the lateral nucleus. Neurons in all three nuclei also projected to the contralateral C_3 gray matter.

Approximately 75% of the neurons received input, often monosynaptic, from the ipsilateral labyrinth; many were inhibited by stimulation of the contralateral labyrinth (commissural inhibition). Commissural inhibition was much more prominent for neurons projecting contralaterally (14/23) than ipsilaterally (7/36). While commissural inhibition has been implicated in the generation of nystagmus (Maeda, Shimazu and Shinoda, 1972) and in the control of vestibulo-ocular reflexes, our results therefore show that it is also involved in control of vestibulocollic reflexes.

The axons of many lateral nucleus neurons projecting to the gray matter of the cervical enlargement branch to the lumbar cord (Abzug, Maeda, Peterson and Wilson, 1974). Neurons in the lateral (44/73) and descending (13/20) nuclei projecting ipsilaterally to the C_3 gray matter also have branching axons, extending to C_{5-7}. 13/30 of these branching axons of lateral nucleus neurons reached the thoracic cord below the enlargement. In contrast to the entire population of neurons projecting to the enlargement, however, only 2 of the lateral nucleus neurons projecting to C_3 had axons that reached lumbar levels. This shows that neurons branching to different spinal cord levels are not homogeneous but instead are divided into at least 2 different populations.

The nature of some vestibular neurons was revealed by experiments such as those of Jankowska and Roberts (1971). Neurons in the vestibular nuclei were fired by glutamate iontophoresis or labyrinth polarization. The synaptic potentials caused by activity of these individual vestibulospinal neurons were studied by intracellular recording from neck motoneurons with the aid of signal averaging. In agreement with earlier stimulation experiments (Wilson and Yoshida, 1969) 4 inhibitory neurons were in the medial nucleus or on the border between the lateral and medial nuclei. One was in the descending nucleus. These 5 neurons were located in areas containing few cells with long axons (0/10 medial nucleus neurons could be activated from C_{5-7}) and none of the 4 neurons tested had such an axon. It may be that inhibitory vestibulospinal neurons do not distribute to more than one cord level.

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1523 SHORT-TERM EFFECTS OF STREPTOMYCIN ON VESTIBULAR NEURONS IN GERBIL. David J. Anderson and Robert O. Andres*. Kresge Hearing Research Institute and University of Michigan, Ann Arbor, MI 48109.

Using our technique for recording from single first order neurons in Gerbil, the short-term effects of streptomycin on vestibular nerve responses were observed. In addition to the usual preparation for Scarpa's ganglion recording in Gerbil, an opening in the bulla was made over the round window on the operated side. Once responses from a small population of horizontal canal neurons have been recorded and confirmed to have spontaneous activity and sensitivity parameters within the range of our normal data base, a neuron is selected for experimentation. Streptomycin is injected into the bulla and enters the innear ear from the middle ear by diffusion through the round window. Following introduction of the drug, the neuron is carefully observed over a period not less than thirty minutes. The typical course of the experiment when performed on a neuron having regular interspike interval statistics is for all of the parameters to remain stable for about twenty minutes followed by a decrease in sensitivity, a decrease in spontaneous activity and changes in discharge pattern toward a more irregular characteristic. Neurons which are irregular at the onset of the experiment remain irregular with decreases in sensitivity and spontaneous activity. At the conclusion of observations on the principle neuron selected for study, more neurons are observed to confirm that the population statistics have shifted to reflect the introduction of the vestibulo-toxic agent. Eight neurons have been tested in this fashion with an additional five neurons begin used as controls.

1524 DEVELOPMENT OF VESTIBULAR NYSTAGMUS IN INFANTS AND CHILDREN. Constance W. Atwell*, Edward M. Ornitz and Elizabeth Eugenie Hartmann*. Pitzer College, Claremont, Ca. 91711 and Dept. of Psychiat., Sch. Med., UCLA, Los Angeles, Ca. 90024.

Developmental comparisons of vestibular nystagmus were made among normal human infants and older children in response to 18 seconds of controlled rotatory acceleration ($10^{o}/sec^{2}$) and approximately 150 seconds of constant velocity stimulation (180^{o}/sec) in total darkness. Measurements of amplitude and duration of individual nystagmus beats during per-acceleratory primary nystagmus, constant velocity primary nystagmus, and secondary nystagmus during constant velocity rotation yielded the following developmental differences: Relative to older children (22-139 months of age), the infants (3-12 months) responded with (1) larger amplitudes per beat during both slow and fast phases of the nystagmus beat; (2) less total duration of both slow and fast phases summed over all beats; (3) higher velocities during both slow and fast phases; (4) fewer beats; (5) less effective time of nystagmus, i.e. from first to last beat of each stimulation period. In general, the same trends were found within the infant sample as a function of age that distinguished the infant group from the older children. The total time course of the infants' response to the rotational stimulation may be characterized by an immediate intense response which is shorter in duration than that of the older children.

1525 EVOLUTION OF OCULOMOTOR RESPONSES TO OPTOKINETIC AND VESTIBULAR STIMULI AFTER UNILATERAL LABYRINTHECTOMY IN HUMANS AND CATS. Luis D. Benítez and Jorge Corvera*. Laboratory of Audio-Vestibular Physiol., Dept. Sci. Res., Natl. Med. Ctr. I.M.S.S. Mexico.

Daily electronystagmographic recordings were made in human subjects and in cats before and after unilateral labyrinthectomy, or (in humans) after differential sectioning of the vestibular nerve. Sinusoidal angular accelerations were used as stimuli, with and without covering the eyes. After surgery, spontaneous nistagmus followed a predictable course and disappeared after 5-10 days. Directional preponderance was maximal during the first 5 postoperative days and disappeared at 8-10 days. Overall magnitude of vestibular responses to accelerations in both directions was severely diminished from about 3 to 25 days. The integrated movement of the eyes was a predictable percentage of the ambient movement, 100% with ambient fixation and 40-50% without. These percentages change in a predictable way after surgery. Considerations are made on the possible relations between these observations and studies of the electrical activity in the vestibular nuclei, as well as the possible role of the efferent system. Possible clinical use of this method will be discussed.

1526 OTOLITH-VISUAL INTERACTIONS IN SINGLE UNITS OF CAT VESTIBULAR NUCLEI. Nancy G. Daunton and Dianne D. Thomsen*. Neurosciences Branch, NASA-Ames Research Center, Moffett Field, CA. 94035.

Recent investigations have described semicircular canal-visual interactions in the vestibular nuclei of monkeys (Henn, et al., Br. Res., 1974, 71, 144-149). These interactions have been related to the illusion of self-rotation (circularvection) found in humans (Young, et al., Acta Otolaryng., 1973, 76, 24-31). The present study was undertaken to investigate the existence of otolith organ-visual interactions.

Single units in the vestibular nuclei of chronic cats, relaxed with Flaxedil and artificially ventilated, were tested in the dark for responses to linear accelerations in fore-aft, right-left, and up-down directions. Units found to respond to one or more of these vestibular stimuli were then tested in the light for responses to a large visual stimulus moving linearly in the fore-aft, right-left, or up-down direction, stimulating both the cat's frontal and its peripheral visual fields, while the cat remained stationary.

The majority of units found to respond to linear acceleration in a given direction also responded to the visual stimulation which simulated actual movement of the cat in the same direction. Thus, a cell which was excited by movement of the cat to the left, would also be excited by movement of the visual stimulus to the right. While the data show that most units were less sensitive to visual stimulation than to vestibular stimulation, some units were found which had higher gains in response to visual stimulation than in response to vestibular stimulation. Results of this experiment provide a possible neurophysiological basis for some of the behavioral aspects of linearvection (illusions of linear self-movement) detailed by Bertoz, et al. (Exp. Br. Res., 1975, 23, 471-489).

1527 THALAMIC COMPONENTS OF ASCENDING VESTIBULAR PROJECTIONS. Mary Day*, Paul Blum, Malcolm B. Carpenter, and Sid Gilman. Depts. Neurol. and Anat., Columbia Univ., College of Physicians and Surgeons, New York, NY 10032.

Vestibular stimulation in cat evokes responses in the anterior suprasylvian (AS) and post cruciate (PC) cortex. To locate the thalamic sites mediating this activity, electrical stimulation was applied to the vestibular nerve, the cortical sites showing maximal responses were defined, and horseradish peroxidase was injected into these sites. After two days survival, the animals were sacrificed and brain sections processed to visualize enzyme reaction products. After AS injection the majority of labeled neurons were located in the dorsolateral portion of the ventral posterolateral nucleus (VPL). Other thalamic nuclei with labeled neurons included ventral lateral, ventral posteromedial (VPM), intralaminal and reticular. A few labeled cells were found scattered in mangocellular portion of medial geniculate body. After PC injection, the labeled cells were found in these same thalamic nuclei, however, labeled cells in VPL were located more rostrally. In separate experiments responses to vestibular nerve stimulation were recorded from thalamus. Evoked potentials were found in these same thalamic nuclei, however, the shortest responses (<3 msec) were found in VPL and VPM. These data indicate that VPL and VPM relays vestibular activity to both AS and PC projection sites in cortex.

(Supported by USPHS Grants NS 11307 and 01538).

1528 ZERO GRAVITY AND DEVELOPMENT: BEHAVIORAL ANALYSES OF KILLIFISH EXPOSED TO WEIGHTLESSNESS IN APOLLO-SOYUZ EXPERIMENT MA-161. Ronald B. Hoffman, Gloria A. Salinas*, and Anwar A. Baky*, NASA-JSC, Houston, TX 77058.

This study focused on the vestibular competence of two groups of Fundulus heteroclitus which were subjected to nine days of weightlessness. The first group consisted of a graded series of embryos representing key developmental stages. Using the strong diving response in this species as a measure of geotaxis, a significant difference ($p < .05$) was revealed in the flight 32 hour-stage (pre-liftoff fertilization time) but not for the 66 hour- and 128 hour-stages as compared with ground controls. The 32 hour-stage at orbital insertion encompassed late gastrulation, neural groove closure, and formation of rudimentary optic vesicles. The detection of this difference occurred when the fish were 6-8 months old. A high correlation ($r = .85$) was found between the number of flight fish in a given tank and the geotaxis score. This finding plus the trends for the scores under different photoperiods suggest a greater sensitization of the flight fish to environmental influences. The second group consisted of 21-day old juveniles which were subjected to light orientation tests soon after recovery. No significant differences were found between the flight and ground controls. Further assessment of the effect of weightlessness on development in the killifish is in progress.

1529 COMPENSATION TO HEMILABYRINTHECTOMY IS TEMPORARILY ABOLISHED BY UNILATERAL LUMBAR OR SACRAL REGIONAL NERVE BLOCK. David W. Jensen. Dept. Neurosciences, Sch. Med., UCSD, La Jolla, CA 92093.

Most of the severe postural asymmetries and incoordination produced by hemilabyrinthectomy disappear with 24 hrs. in healthy guinea pigs, thereby demonstrating vestibular compensation (review by Schaefer & Meyer, Hndbk. Sens. Physiol., VI/2, 1964). The following results strongly imply an increased importance of sensory input from local posterior levels to the spinal cord in maintaining symmetrical posture in guinea pigs that have compensated to hemilabyrinthectomy. A protractor device was used to measure the attitudes of lateral head deviation, trunk curvature and longitudinal twisting in blindfolded intact and compensated animals. A unilateral Xylocaine block immediately dorsolateral to the vertebral column at L-1, or in the first sacral intervertebral foramen in intact animals produces only localized anesthesia, or especially in the sacral injections, an additional local paresis, but no perceptible changes in the symmetry of the measured attitudes. However, the same injections produced in addition in compensated animals a striking appearance or marked enhancement of asymmetry of the aforementioned attitudes. In compensated animals these evoked asymmetries from all injections ipsilateral or contralateral to the previous hemilabyrinthectomy were in direction and occasionally in amplitude the same as for the original hemilabyrinthectomy. The nerve block symptoms appeared within 3-5 min., lasted for more than an hour, and then returned to the pre-injection state.

1530 INFLUENCE OF THE CONTRALATERAL LABYRINTH ON RESTING AND DYNAMIC ACTIVITY OF CAT VESTIBULAR NUCLEUS CELLS. Charles H. Markham, Toshiaki Yagi* and Ian S. Curthoys*. Dept. Neurol., Sch. Med., UCLA, Los Angeles, CA 90024.

Brain stem vestibular neurons were recorded extracellularly in alert cats with C-1 spinal transsections. Neuronal and field responses to natural and electrical stimulation of the labyrinth were determined before and after inactivation of the contralateral labyrinth. Brain stem vestibular neurons connected to the horizontal canal showed a moderate to marked increase in resting rate (means changing from 21 to 52 spikes/sec, n=72); a marked increase in background resting activity in the vestibular nuclei; and a reduction in sensitivity at $5^{o}/sec^{2}$ angular acceleration from about 6 to 3 extra spikes/sec/deg/sec^{2}, the latter being similar to the mean sensitivity of the first order afferents. The amplitude of the N-1 response after contralateral VIII nerve cutting was 50-100% greater, with the amplitude difference more marked at higher stimulus intensities. Other alterations in vestibular nuclear activity will be presented.

1531 CONFIGURATION OF THE CUPULA DURING ENDOLYMPH PRESSURE CHANGES.
J.W. McLaren* and D.E. Hillman. Div. of Neurobiol., Univ. of Iowa, Iowa City, Ia. 52242.

The cupula has been described as a transparent gelatinous structure supported on the crista where it forms a partition across the semicircular canal ampulla. Functionally, it has been demonstrated that endolymph movement displaces the cupula as if it were hinged on its receptor cell base. In this study, a new technique has been developed for observing displacement of the bullfrog cupula in response to endolymph pressure changes. A micropipette was inserted through the cupula of the horizontal canal, from the apex to the crista, and withdrawn with simultaneous injection of a dye tract. Pressure differences across the cupula were then generated by compression of the semicircular canal and the resulting cupular displacement was recorded by high speed cinematography. The time course for displacement of various regions of the cupula was analyzed and graphed from consecutive single frames. The results showed that the cupula remained fixed around its perimeter where it contacted the ampullary wall and moved as a diaphragm with maximum displacement at a central zone rather than the traditionally described apical region. During a pressure change cupular displacement originated near the center of the crista and produced a shearing movement, across the subcupular space, which was limited by the crista attachment. Hence, a relatively small volume displacement of the endolymph may be required for minimal receptor cell activation. From the center of the crista, displacement spread to the ends of the crista as well as to the center of the cupula. The "diaphragm cupula" arrangement thus suggests a mechanism of graded reception and a much more sensitive means of endolymph movement-receptor cell coupling than the traditional "swinging gate cupula". Supported by PHS Grants NS-09916 from NINCDS and GM-00225 from NIGMS.

1532 ASYMMETRY OF SEMICIRCULAR CANAL-EXTRAOCULAR MUSCLE FUNCTION IN FLATFISH.
Christopher Platt. Dept. Elec. Eng. & Comp. Sci., Univ. of California, Berkeley, California 94720.

Flatfish are unique vertebrates in having both eyes on one side of the head, adopting a normal adult posture lying on the "blind" side. Their eyes maintain a nearly horizontal gaze, shifting the visual relations to the body by 90° relative to other vertebrates, requiring changes in compensatory eye movements to head rotation. For example, in other vertebrates head rotation around the dorsoventral axis elicits ocular compensation so that one eye swings nasally, the other temporally around its dorsoventral axis. But in flatfish the morphologically identical stimulus swings the head up and down in the vertical plane, and the compensatory response is a rolling of both eyes together around their optic axes.

This behavioral change could result from peripheral changes in receptors or muscles, or alternatively from central changes in neural connections. Morphological observations indicate that peripheral changes are not present. 1) The labyrinths have not rotated as the eyes have, and appear normally oriented in the head, so the "horizontal" canals lie vertically. 2) The six eye muscles have not changed positions, and retain normal origins and insertions from orbit to eyeball. 3) Innervation of the eye muscles has not changed; nerves III, IV and VI course normally from the brain surface out to the correct muscles. 4) Hair cell orientations within the canal ampullae have not changed; SEM shows the normal "utricle-facing" kinocilia in the "horizontal" ampullae, and "canal-facing" kinocilia in the anterior and posterior ampullae.

Thus, unlike other vertebrates, the flatfish produces bilaterally symmetrical neuromuscular output from asymmetrical stimuli to the vestibular receptors, and conversely. The functional change apparently is not at peripheral levels but must occur in the central pathways, direct and indirect, between the vestibular nuclei and extraocular muscle nuclei.

1533 EFFECTS OF TILT ON THE RESPONSES OF PRECEREBELLAR RETICULAR NEURONS TO SOMATOSENSORY VOLLEYS. O. Pompeiano, J.D. Coulter and T. Mergner*. Istituto di Fisiologia Umana, Cattedra II, Università di Pisa, Italy.

The activity of neurons located in the precerebellar lateral reticular nucleus (LRN) were recorded in decerebrate cats and their responses to lateral tilt of the animal studied. Units which were activated antidromically on electrical stimulation of the cerebellar anterior lobe showed steady increases in discharge rate during tilt in one direction, while tilt in the opposite direction resulted in a decrease in discharge rate. The units affected by tilt received inputs from both ipsilateral and contralateral forelimb and/or hindlimb nerves. The responses of LRN neurons to stimulation of cutaneous and high threshold muscle afferents were either increased or decreased and sharpened during tilt, in parallel with the changes in the spontaneous discharge rate induced in the same units by tilt. The changes in magnitude of the responses of LRN neurons to somatosensory volleys were clearly related to the degree of tilt. Thus macular stimulation may, in addition to producing tonic changes in spontaneous firing, also modify the phasic responses of these neurons to peripheral nerve stimulation. The interaction between macular input and somatic input within the LRN neurons may produce fine adjustments of the corticocerebellar activity, thus leading to possible changes in the reflex response of the vestibulospinal neurons to macular labyrinthine stimulation.

1534 VESTIBULAR-SPINAL RESPONSES AS A FUNCTION OF ZERO-G. Millard F. Reschke*, D. J. Anderson, M. Moore*, and J. L. Homick*. Neurophysiology Section, NASA-Johnson Space Center, Houston, TX. 77058. (SPON: M. Igarashi, Baylor College of Medicine, Houston, TX. 77058.)

Measurements of postflight postural ataxia following Skylab have indicated that prolonged exposure to weightlessness can effect those vestibulo-spinal systems responsible for equilibrium and auto-stabilization of the body. To further investigate this finding vestibulo-spinal reflexes have been studied during parabolic flight. NASA's KC-135 airplane was used to provide vestibular (otolith) stimulation. Subjects were placed in a supine position and restrained in a litter so that accelerations were perpendicular or through the x-axis of the body. Variable accelerations ranging from approximately 1.8 to 0-g (weightlessness periods lasted as long as 25 seconds) were obtained by flying the aircraft in a roller coaster fashion. Soleus/spinal H-reflex testing procedures were used in conjunction with the acceleration stimulus to assess changes in postural muscles as a function of variable g load on the otoliths. Results indicate that weightlessness induces a potentiation of the H-reflex, which lasts throughout the period of 0-g, and that hyper g results in an inhibition of this response. However, the percentage of soleus/spinal motoneurone pool facilitation or inhibition varies across subjects. This variability observed across subjects relates with the further observation that these subjects have also demonstrated varying degrees of tolerance to motion sickness both during ground based testing and parabolic flight.

1535 ANALYSIS OF THE DYNAMIC RESPONSES OF CAT SEMICIRCULAR CANAL AFFERENTS Robert H. Schor, David L. Tomko, Dennis P. O'Leary and F. O. Black Depts. of Otolaryngology and Pharmacology, Univ. of Pittsburgh, Sch. of Med., Eye and Ear Hospital, Pittsburgh, PA 15213

Dynamic response characteristics of mammalian semicircular canal afferents have been estimated previously using constant angular acceleration of sinusoidal rotations. Alternatively, a pseudorandom binary sequence (PRBS) of rotational acceleration can be used as a bandlimited white noise input for estimating dynamic response properties of semicircular canals. Cross-correlation of the PRBS stimulus with the neuronal response yields the impulse response function, which, in addition to estimating linear characteristics, may be used to predict the response of the system to other inputs.

Responses of single units in the vestibular nerve of barbiturate-anesthetized cats were studied using rotations in the plane of the horizontal semicircular canal. Dynamic response properties of each unit were estimated from the response to three to ten periods of PRBS (test protocols of 4 to 8 minutes) with bandwidths of about two decades centered near 0.1 Hz. The units were also studied with velocity trapezoids with constant accelerations of from ten to forty seconds and with velocity sinusoids of from 0.02 to 1.0 Hz. Responses to trapezoids were predicted, for comparison with experimental results, by convolving the calculated impulse response from the PRBS with the trapezoid acceleration profile. Experimental responses to sinusoids were predicted by estimating the Fourier amplitudes and phases of the impulse response at each stimulus frequency.

On the basis of preliminary results, the amplitudes of experimentally determined sine wave responses were predicted relatively well from impulse responses except near the high frequency limits of the noise stimulus. We conclude that the PRBS technique provides an efficient estimation of the power spectrum of this system. (Supported by NIH grants NS 12494-02 and NS 12308.)

1536 HUMAN EYE TRACKING DURING VERTICAL MOTION.† Thomas R. Vidic*, John S. Barlow, Charles M. Oman*, John R. Tole*, Alfred D. Weiss*, Laurence R. Young. Dept. of Neurology, Mass. General Hosp., Boston, MA. 02114, and Harvard Medical School, Boston, MA. 02115, and Dept. of Aeronautics and Astronautics, Mass. Inst. Tech., Cambridge, MA. 02139.

To investigate the possible role of the otolith organs in tracking of actual and of imagined stationary fixation points during body motion, 9 normal subjects underwent repetitive vertical displacements (54 cm,=30° eye movement with fixation at 1 meter) in a constant-velocity, automatically reversing (2 sec each phase) hoist-operated chair.

During attempted tracking of the imagined fixation light with eyes open in total darkness, eye movements (EOG-monitored) of subjects without prior experience in the moving chair were initially very erratic (primarily saccadic), with only a low-amplitude smooth component. After extinction of the fixation light during a run, a few cycles of relatively smooth tracking ensued before the more saccadic behavior reappeared, which contrasts sharply with an earlier finding (by A.W. and J.B.) of persistent smooth compensatory tracking by fixating subjects rotated in a Bárány (swivel) chair in the dark. A brief flash of the fixation light at the middle of each excursion increased both amplitude of eye movement and the smooth tracking content. After repeated trials that included some tracking in the light, subjects showed an increased component of smooth tracking of the imagined target in darkness.

To attempt to isolate vestibular influences, we are testing patients with labyrinthine function bilaterally absent; our first patient showed only minimal smooth eye movements for a visible fixation light, and none in darkness, or with the intermittently illuminated fixation light, even after practice.

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1537 PHYSIOLOGICAL IDENTIFICATION OF VESTIBULAR EFFERENTS AND THEIR RELATIONSHIP TO THE GOLDFISH MAUTHNER CELL. Steven J. Zottoli and Donald S. Faber. Res. Inst. on Alcoholism, 1021 Main Street and Dept. Physiol., SUNY at Buffalo, Buffalo, N.Y. 14203.

Intracellular recordings of responses to ipsilateral vestibular nerve stimulation have been obtained from neurons electrically inhibited by the goldfish Mauthner cell (M-cell). This inhibition is characterized by a "passive hyperpolarizing potential" (PHP) produced by a M-cell antidromic spike following spinal stimulation (Faber and Korn, Science; 179: 577, 1973). Some PHP neurons are found 250-450 μm lateral to the M-cell's axon hillock and close to the cell's lateral dendrite; these neurons are antidromically excited by VIIIth nerve stimulation (latency range 200-500 μsec). They are not afferents since they receive mono- and polysynaptic excitatory inputs both from the VIIIth nerve and following M-cell antidromic activation. These results suggest the neurons are rather vestibular efferents. Approximately 90% of these PHP neurons also exhibited graded short latency depolarizations when the VIIIth nerve stimulus was below threshold for antidromic invasion. The latency difference between the antidromic spikes and the graded depolarizations was always less than 200 μsec, which is too short for a chemical synaptic delay, indicating that the efferents are electrotonically coupled, either directly or through terminals of vestibular afferents.

PHP neurons have been shown to be inhibitory to the M-cell (Korn and Faber, J. Neurophysiol; 38: 452, 1975). The evidence that some of these neurons are also vestibular efferents and are activated synaptically by the M-cell's collateral network suggests their activation would depress M-cell excitability both postsynaptically and through a suppression of the afferent input to that cell. (Supported by NIH Grant NS-12132-02 and Postdoctoral Fellowship No. F32 NS 5282-01 to S. Zottoli).

Vision

1538 THE ROLE OF THE GENICULO-CORTICAL SYSTEM IN FORM VISION: AREA 17 AND 18 AND CONTOUR ACUITIES. M. A. Berkley, J. M. Sprague, and D. S. Warmath*. Dept. Psychol., Florida State Univ., Tallahassee, FL. 32306 and Dept. Anat. Sch. Med., Univ. Pennsylvania, Philadelphia, PA. 19174.

Evidence gathered from the study of the striate cortex in the monkey strongly suggests that this structure is critical for the "seeing of shapes". Although electrophysiological evidence gathered from the cat is consistent with such a conclusion, neuro-behavioral studies are not in that several investigators have found minimal defects of form vision in tests of cats lacking "striate cortex", e.g., Doty, 1971; Sprague, et al., 1973). Since it has not been possible to specify the specific cue being used in most form discriminations, e.g., overall shape, corner, border, contrast distribution, it is not clear from previous ablation studies whether a different cue is being used in postoperative form discriminations, or the same cues are being used by the remaining portions of the system. To cope with this problem and thus elucidate the role of "striate cortex" in form vision of cats, we used stimuli in which one dimension of the stimulus could be varied. Using a two choice discrimination paradigm, thresholds were determined for size (gratings), parallelness (parallel vs. non-parallel lines), contour alignment (vernier offset), and angularity (polygon figures) pre- and postoperatively in cats. This procedure permitted accurate specification of the controlling cue in a complex visual display.

Animals with a total loss of area 17, up to 90% of area 18 and with and without marginal infringement of area 19 showed about a 20% reduction in grating acuity, a three-fold increase in parallelness and angularity thresholds (3°-5° preop-12°-15° postop), but a total loss of the ability to discriminate offset line targets (vernier offset). All animals were able to learn classic form discriminations postoperatively but at a slower rate than normal. Comparisons are being made with animals with lesions of other portions of the visual system.

Considering the differences in the geniculo-cortical systems of primates and cats, the results of the present study are consistent with the hypothesis that in the cat, unlike the monkey, there are parallel geniculo-cortical and extrageniculo-cortical pathways capable of processing spatial information of some detail. They also suggest, however, that certain stimulus dimensions can only be processed by the geniculo-cortical system.

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1539 UNIT ACTIVITY IN THE INFEROTEMPORAL CORTEX OF RHESUS MONKEY DURING THE PERFORMANCE OF VISUAL DISCRIMINATION TASKS. David J. Braitman* and William A. Wilson, Jr. Depts. of Biobehavioral Sciences and Psychology, University of Connecticut, Storrs, Ct. 06268. U.S.A.

Unit activity was recorded from the inferotemporal cortex of three rhesus monkeys while they performed a series of four successive discrimination tasks. Monkeys were trained to press a start button when it was lit in order to initiate the brief presentation (0.01 msec) of one of two discriminative stimuli 500 msec later. The monkey was allowed 1 sec to press the right or left side of a panel, depending upon which stimulus was back-projected upon it. A correct response was followed by liquid reinforcement 110 msec later. The discriminanda were checkerboard patterns (either red and black or green and black) which subtended 13° 25' of arc on the retina. Individual checks in the checkerboards were either 2° 14' or 33' in visual angle. During some discriminations (eg. red large checks vs. green large checks) monkeys were required to attend to color in order to respond correctly. In other discriminations they might be required to discriminate the check size (eg. red large checks vs. red small checks). Thus by proper sequencing of tasks either the relevant dimension to be discriminated or one of the pair of stimuli could be held invariant between successive discriminations. During each trial data were examined before and after the stimulus presentation, before the response, and after the reinforcement.

The activity of 131 (92%) of 143 inferotemporal units was altered by the presentation of the visual discriminanda; 113 of these cells (86%) exhibited a statistically significant increase in firing after presentation of a stimulus while the remainder gave an inhibitory response. Almost all of the responsive units (89%) showed a change in firing within 50 to 149 msec after the presentation of a stimulus; over half (60%) of these exhibited a significant difference in the rate of firing to the two discriminanda presented, while the remainder showed the same response to the stimuli.

In essentially all units, neural activity was more highly correlated with the stimulus than with the behavioral response. The stimulus-locked activity of some inferotemporal neurons did appear to reflect the response, left or right panel press, which was required by the stimulus.

Over half the responsive neurons that were monitored during more than one discrimination task were sensitive to the stimulus dimension attended to when the stimulus or relevant dimension remained invariant between tasks. Thus, six out of the ten neurons recorded during a shift in the relevant dimension from color to check size or check size to color exhibited a difference in poststimulus neural activity for the stimulus that was the same colored checkerboard pattern before and after the shift. Three neurons were recorded from when the stimuli were altered by changing the check size, although the relevant dimension (color) was left the same; two showed an invariant response to the altered stimuli and one gave the same response to one of the altered stimuli but a different response to the other. Other neurons gave the same response to a given stimulus even though the relevant dimension in the colored checkerboard pattern was shifted.

These results suggest that some inferotemporal neurons are involved in selective attention or cue abstraction while others function in the integration of multidimensional visual stimuli.

1540 RELATIONS BETWEEN THE OPTIC TECTUM AND BASAL GANGLIA IN THE PIGEON. N. Brecha*, S. P. Hunt*, H. J. Karten. Dept. of Psychiatry., Sch. Med., S.U.N.Y. at Stony Brook, Stony Brook, NY 11794.

The optic tectum receives an obvious and well defined "visual" input from the retina, visual Wulst and associated subtectal nuclei, isthmi, pars parvocellularis (Ipc), n. semilunaris (SLu), n. pretectalis (PT) and n. lentiformis, pars magnocellularis (LMmc). However, a wide variety of tectal afferents have been described, including the presence of a rich catechol amine (CA) plexus throughout the tectal cortex.

In order to determine the possible presence of these proposed afferents, HRP has been injected into the superficial portions of the optic tectum. Following 24-48 hours survival brains were processed according to the protocol of LaVail and LaVail (JCN 157: 303, 1974). In addition to labelled cells within the cell groups outlined above, labelling was noted in the ipsilateral SpL and the nucleus tegmenti pedunculo pontinus (TP). To date no labelled cell bodies have been found within the n. rotundus, n. spiriformis medialis or the locus coeruleus (LoC).

Numerous SpL neurons were heavily labelled. Only scattered heavily labelled cells were found in TP. Both SpL and TP receive a massive and seemingly exclusive input from the palaeostriatal complex (PC) (equivalent to basal ganglia in mammals) via the ansa lenticularis. Fluorescent studies for CA revealed a high concentration of CA neurons in TP. No fluorescent cells were identified in SpL. The differential effect of lesions in TP and LoC on tectal and PC, CA's were compared.

Injections of tritiated amino acids into SpL demonstrated that the nucleus projects heavily upon all layers of the optic tectum, including retinal recipient as well as efferent layers.

These findings reveal the existence of a major input from the basal ganglia via SpL to the tectum. The precise relationship of SpL inputs upon the ascending tectal pathway or the descending (premotor) pathway is unknown. Further studies are needed to determine whether seperate populations of tectal neurons exist that project either into the ascending visual channel or the descending pathways and whether PC has a direct influence on visual processing within the tectal cortex.

1541 RECEPTIVE-FIELD STRUCTURE OF X- AND Y-CELLS IN THE CAT LATERAL GENICULATE NUCLEUS. Jean H. Bullier* and Thomas T. Norton. Duke University, Durham, N. C.

To study the receptive-field structure of X- and Y-cells in the cat LGN we have 1) made spatio-temporal maps of the excitatory regions, 2) studied the inhibitory effect of the surrounding region on the excitatory response of the central region. Spatio-temporal maps were made by presenting a small flashing spot in fifty successive, overlapping positions across the receptive field, recording the response of the cell, and displaying the fifty successive responses as a raster pattern. Fig. 1 shows the response of an X-cell and Fig. 2 the response of a Y-cell. Each mark represents a spike, each successive horizontal row of marks represents the response to onset (500 msec on) and offset (500 msec off) of the spot at successive positions. Time is thus represented on the abscissa and space on the ordinate. As shown in these figures, responses from the central excitatory region (represented by the central rows in the left half of each figure) are distinct for X- and Y-cells. For X-cells the central region consists of a very sensitive area which gives a spatially homogeneous response and has well-defined borders. For Y-cells the central excitatory domain consists of two overlapping regions: a large region where a strong transient response can be elicited and a smaller central region where the transient part is followed by sustained firing. Both regions have more gradual borders than the X-cell central region.

To study quantitatively the topography and the strength of the inhibitory surrounding regions we used centrally located spots whose dimensions were increased by small increments to cover progressively the whole receptive field. The response F of the cell to each stimulus size R was estimated by averaging the responses to several presentations. The incremental sensitivity S(R) across the receptive field was computed as: $S(R)=dF/(2\pi RxdR)$, where dF is the difference between the response to a spot of dimension $R+(dR/2)$ and the response to a spot of dimension $R-(dR/2)$. Plots of this incremental sensitivity versus spot dimension provide another characterization of the receptive-field structure: zones of positive sensitivity correspond to excitatory regions and zones of negative sensitivity correspond to inhibitory regions. Such plots show very different profiles for X- and Y-cells: for X-cells the transition between excitatory and inhibitory regions is very steep and the central excitatory region is surrounded by a strong inhibitory region (the classical surround). For Y-cells the sensitivity is lower in the central region, and the transition to the surrounding inhibitory region is more gradual. The inhibitory region is much weaker than for X-cells and is located outside the larger area where the transient excitatory response can be seen on the spatio-temporal map. We conclude that, at the LGN level, X- and Y-cell receptive field structures are strikingly different.

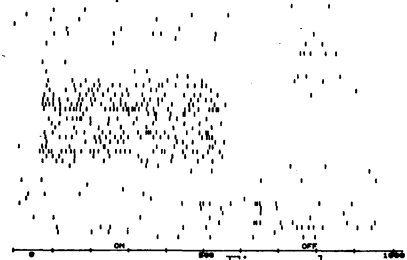

Figure 1

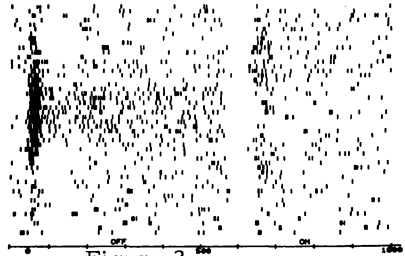

Figure 2

1542 BILATERAL PROJECTION OF THE CENTRAL RETINA OF THE MONKEY DEMONSTRATED WITH HORSERADISH PEROXIDASE NEURONOGRAPHY. A. H. Bunt, D. S. Minckler, and G. W. Johanson. University of Washington, Seattle, WA 98195.

In the primate, ganglion cells (GC's) of the temporal retina project ipsilaterally and those of the nasal retina, contralaterally into the optic tract. The vertical meridian passing through the fovea defines the border between these two populations of GC's and has been demonstrated in four Rhesus monkeys after unilateral injection of horseradish peroxidase (HRP) into the dorsal lateral geniculate nucleus (dLGN) and examination of the pattern of retrograde labeling in flat mounted retinae of those GC's projecting to the injected side. A median vertical strip of overlap approximately 1° wide in which ipsi- and contralateral projecting GC's intermingle was found, confirming the report by Stone et al., (1973, J. Comp. Neurol. 150:333). In addition, occasional extrafoveal labeled GC's were found as far as 2° from the vertical meridian in the otherwise unlabeled hemiretinae. These GC's were not numerous and had somata of all sizes, suggesting that they do not constitute a separate class of GC's as found in the temporal retina of the cat. In contrast to the description by Stone et al., ('73), the strip of vertical overlap did not show a constant width through the fovea, since mixing of labeled and unlabeled GC's was found in a band approximately 1/2° wide along both the nasal and temporal rims of the foveal pit. Beyond this 1/2° rim, the appropriate hemiretina was either completely unlabeled, or contained virtually every GC labeled on the side projecting to the injected dLGN. The scattered labeled GC's rimming an otherwise unlabeled hemifovea represent a possible anatomical basis for the phenomenon of "macular" or "foveal sparing" in which unilateral damage to the occipital cortex produces homonymous hemianopsia with sparing of a small island of centralmost vision extending about 1° from the midline. From this study, it is not possible to define the receptive fields or specific photoreceptor connections of the GC's labeled with HRP, so that at the present time quantitative correlations cannot be made between the numbers of GC's lying on the affected side of the fovea and the extent of preservation of visual function as measured clinically in this pathologic condition. The presence of HRP-labeled GC's rimming the fovea in its entirety is compatible with the sequence of foveal development in late prenatal life. Thus, after lateral displacement both nasally and temporally of GC's which initially lay in the median vertical overlap strip of 1°, in the adult retina a strip approximately 1/2° wide around the perimeter of the foveola should contain a mixture of ipsi- and contralaterally projecting GC's. The total population of GC's beyond this 1/2° band should be completely ipsi- or contralateral in their projection patterns, as is observed. By this developmental sequence, widening of the 1° of vertical overlap to a total of 3° at the fovea is achieved, as was postulated by Morax in 1919.
--Supported by NIH grants EY01311 and EY01756.

1543 STUDIES OF BINOCULAR COMPETITION IN THE DEVELOPMENT OF VISUAL PATHWAYS OF THE TREE SHREW. V.A. Casagrande, T.T. Norton, R.W. Guillery and J.K. Harting, Dept. of Anat., Vanderbilt Univ., Nashville, Tenn.; Dept. of Psychology, Duke University, Durham, N.C.; Dept. of Anatomy, Univ. of Wisconsin, Madison, WI.

Several recent studies have suggested that morphological changes observed following monocular deprivation can be explained by a developmental competitive interaction between the pathways from the two eyes.

In the present study we have investigated retinofugal projections, geniculate cell sizes and the distribution of geniculocortical axons in tree shrews raised from birth with one eye sutured.

Retinal projections were investigated following injections of ^{3}H proline into either the normal or lid sutured eye in deprived tree shrews and into one eye of normal tree shrews. No evidence was found for changes in the organization of the retinofugal pathways. In particular, there were no changes in the laminar distribution of retinogeniculate axons.

Measurements of cell sizes within the lateral geniculate nucleus showed that cells within all six deprived layers of the binocular segment, with the exception of those within layer 3, were smaller than the normally innervated cells. No assymetry was found for cells within layer 3 or for cells in the monocular segment. Since layer 3, which shows no change,is bordered on both sides by layers innervated by the same eye, perikaryal changes may be a function of the proximity of cells that are competing with each other.

Comparisons with measurements made in normal animals indicate that non-deprived cells within the ipsilateral geniculate layers (1 and 5) show some hypertrophy.

Measurements made following unilateral enucleation showed changes in all geniculate layers in both the binocular and monocular portions of the nucleus, suggesting that monocular deprivation and enucleation involve different mechanisms.

We also have investigated changes in the organization of geniculocortical pathways by 1) by placing symmetrical laminar lesions limited to layer 5 in lid sutured animals and comparing the pattern of degeneration within the visual cortex and by 2) studying the transsynaptic transport of ^{3}H proline within the visual cortex following intraocular injections into either the deprived or normal eye of lid sutured tree shrews.

Little or no difference was found in the resulting pattern of degeneration within layer IV of area 17 following the lesions of either deprived or normal layer 5 cells; the degeneration occupied the deepest one-fourth to one-third of layer IV. The autoradiographic picture resembled that reported for normal tree shrews (Hubel, '75; Casagrande and Harting, '75) no matter which eye was injected.

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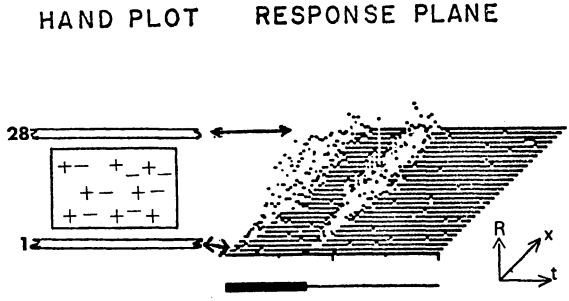
1544 A SPATIO-TEMPORAL COMPARISON OF CORTICAL AND LGN RESPONSES IN THE CAT. Mark C. Citron* and Robert C. Emerson*(SPON: J.L. Brown). Center for Visual Science, University of Rochester, Rochester, NY 14627.

Receptive field properties of both simple and complex cortical cells were studied in the unanesthetized cat using computer controlled static presentations of bright bar stimuli over 28 closely spaced positions in the receptive field. Single unit responses were displayed as a "response plane," a 3-D plot of the averaged responses for a cell as a function of time and location of the stimulus in space.

The responses of complex cells in area 18, characterized by transient excitatory and inhibitory components, were invariant across the receptive field (see Fig.). The resulting response planes were surprisingly similar to those obtained from Y cells in the LGN. The response planes of simple cells were similar to those obtained from X LGN cells although many also contained elements found in Y cell response planes. These new results provide physiological evidence for a functionally significant direct connection from Y LGN cells to complex cortical cells as has been previously suggested. Simple cells appear to receive mixed input, with X LGN connections predominating. Supported by EY 01440.



1545 TOPOGRAPHICAL PROJECTIONS OF THE PRE-STRIATE CORTEX TO THE LATERAL PULVINAR AND INFERIOR PULVINAR IN THE MACAQUE MONKEY. Barry Davis and L.A. Benevento, College of Medicine, University of Illinois Medical School, Chicago, Illinois 60680

Our previous autoradiographic studies (Brain Res., 1976) of pulvino-cortical projections have shown that the projections of the inferior pulvinar (PI) to the pre-striate cortex (Areas 18 and 19) are organized topographically according to the central representation of the visual hemifield in the thalamus and cortex. In addition, these results indicated that points in the adjacent lateral pulvinar (PL) also have this organization in terms of cortical projections. It was of interest, therefore, to determine if there were cortico-pulvinar projections related to the representation of the visual hemifield in the Macaque monkey. 0.3-0.5 µl injections of tritiated proline and leucine were made in the pre-striate cortex of 13 hemispheres of 9 monkeys. The different injection sites were placed in the dorsomedial, dorsolateral and ventro-medial and ventrolateral aspects of pre-striate cortex which is bounded by the parieto-occipital, lunate and inferior occipital sulci caudally, and by the superior temporal and posterior middle temporal sulci rostrally. Injections placed in the dorsomedial pre-striate cortex resulted in a dense concentration of grains in the dorsal portion of PI. Injections placed more ventrally in dorsolateral pre-striate cortex (dorsal portion of pre-occipital gyrus) resulted in a dense focus of grains in a more ventral portion of dorsal PI. Injections placed in the foveal region of pre-striate cortex (near the intersection of the lunate and inferior occipital sulci) resulted in a dense focus of grains located at the dorsolateral border of PI located adjacent to PL and adjacent to the dorsal lateral geniculate nucleus more rostrally. Injections placed in ventrolateral pre-striate cortex (below the inferior occipital sulcus) resulted in a dense focus of grains in the most ventral portion of PI. These results would indicate that the pre-striate projections to PI are organized according to the representation of the visual hemifield. In addition to the grains found in PI, grains were also found in PL. In general, when grains were found in PI, grains were also found in the immediately adjacent PL. However, there were two notable exceptions. First, cortical injections also produced another focus of grains in a portion of PL which was removed from PI. The topography was the same as that found in PI. That is, dorsal pre-striate cortex projects to dorsal PL and ventral pre-striate cortex projects to ventral PL. These findings may indicate another representation of the visual hemifield in PL which is different than that shared by PI and adjacent PL at their borders. Secondly, the results indicate that the foveal representation includes a large portion of PL since grains found in PL after an injection of foveal pre-striate cortex extended into the caudal pole of the pulvinar and extended more caudally than the location of grains in PI. In these cases, dense grains were also often found in the caudate nucleus, reticular nucleus of the thalamus and the superior colliculus.

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1546 STEREOSCOPIC VISION IN THE FALCON (FALCO SPARVERIUS). Robert Fox, Stephen W. Lehmkuhle*, and Robert C. Bush*. Department of Psychology, Vanderbilt University, Nashville, Tennessee 37240.

Many features, e.g., semidecussation of the optic tract, of the visual system of binocular mammals seem to have evolved expressly for the promotion of stereopsis, thereby rendering plausible the assumption that these animals possess the capacity for stereoscopic depth perception. The absence of these features, e.g., complete decussation of the optic tract, in binocular nonmammalians has been interpreted to mean that stereopsis may be an exclusive product of mammalian vision. But recent anatomical investigations of nonmammalian visual systems (e.g., Karten, Hodos, Nauta, & Revzin, Journal of Comparative Neurology, 1973, for owl and pigeon) have revealed pathways that permit the wholesale interaction between eyes generally considered essential for stereopsis. Motivated by these anatomical data we have carried out a behavioral test of stereopsis in the American kestrel, a small falcon known to have special adaptations, e.g., temporal foveae, for binocular vision. The method of testing was a spatial two-choice discrimination task that required the bird to fly to a stimulus display containing a stereoscopic or cyclopean square. Selecting the display with the square (the correct stimulus) produced a food reward; selecting the display without the square (the incorrect stimulus) yielded no reward. The stereopsis displays were random-element stereograms composed of a matrix of minute red and green dots continuously generated on modified color television receivers. To provide dichoptic stimulation the anaglyph method was used, wherein image separation is produced by requiring the observer to view the display through chromatic filters. To this end, the bird was trained to examine the displays and fly toward them while wearing a helmet-spectacle frame device that placed red and green filters before its eyes. Several of the attributes of the stereoscopic stimulus could be quickly changed via the electronic systems generating the displays. Variations in attributes included apparent motion of the stereoscopic form in either the X or the Z axis and changes in both the magnitude and sign of the disparity. During the first phase of training, monocular cues were gradually removed. In the final phase of testing all stereogram elements appeared to be in apparent motion as a consequence of their continual replacement every frame by a random generator; the cyclopean square appeared to be stationary and in front of the background plane. Under these conditions, stereopsis provides the only basis upon which a discrimination can be made. The performance of the bird varied systematically, over repeated sessions, with the magnitude of disparity. Performance reached the level of 80% correct for the largest disparity obtainable and declined to 50%, chance level, for the smallest disparity. In control sessions, where both eyes were covered with filters of the same color, discrimination fell to chance. Since only an incomplete set of disparity values could be generated, only an approximate estimate of stereoacuity could be obtained; yet the data are sufficient to indicate that falcon stereoacuity is on the same order of magnitude as that obtained from humans in the same apparatus.

From these results we are led to conclude that the falcon has the capacity for stereoscopic depth discrimination. Moreover, it must possess the more complex form of stereopsis attendant to random-element stereograms known as global stereopsis (on this point, see Julesz, 1971, and Bishop & Henry, 1971). Up to now global stereopsis has been demonstrated only in humans and in rhesus monkey. Our data are consistent with the anatomical evidence for interocular interaction in birds, and support the general hypothesis that stereopsis may be an intrinsic property of all animals whose visual systems permit both eyes to view simultaneously a common segment of visual space.

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1547 DESCENDING PATHWAYS FROM THE MONKEY SUPERIOR COLLICULUS.
J.K. Harting. Department of Anatomy, University of Wisconsin, Madison, WI. 53706.

For the present analysis the autoradiographic tracing method was used to identify the various descending tectofugal pathways and their targets in seven rhesus monkeys.

The autoradiographs reveal two major descending pathways i.e. the crossed tectospinal tract (or predorsal bundle) and the ipsilateral tectopontine, tectobulbar tracts. Axons which comprise the predorsal bundle arise exclusively from the deeper tectal layers (i.e. ventral to the stratum opticum), cross within the dorsal tegmental decussation and descend within the brainstem in a position slightly lateral to the midline. The most rostral and quite impressive target of the predorsal bundle is the nucleus reticularis tegmenti pontis. While transported protein is restricted to only the dorsal medial portion of the nucleus rostrally, at more intermediate levels an extensive medial sector of the nucleus contains label. At these same levels, scattered labeled axons pass laterally to end within the nucleus reticularis pontis oralis. Further caudally, at approximately the level of the superior olivary complex, numerous labeled axons leave the predorsal bundle, and pass dorsally and laterally to reach the dorsal regions of nucleus reticularis pontis caudalis. At more caudal brainstem levels a dense locus of lable lies immediately ventral to the abducens nucleus. In one experiment label was observed within the most rostral portion of the nucleus itself. Further caudally, scattered axons continue to pass from the predorsal bundle to end within the medial portions of nucleus reticularis pontis caudalis. At caudal medullary levels the majority of the labeled axons comprising the predorsal bundle pass ventrally to end within subnucleus b of the medial accessory nucleus of the inferior olive.

The ipsilateral pathways also arise primarily from the deeper tectal layers and course laterally and ventrocaudally to terminate within the parabigeminal nucleus, the mesencephalic reticular formation, the dorsal lateral pontine gray (in several discrete patches) and sparsely within the nucleus reticularis tegmenti pontis. Several regions within the most lateral portion of the mesencephalic reticular formation receive what appear to be right angle collaterals from the descending bundles of labeled axons. Other ipsilateral targets are the cuneiform nucleus, the external nucleus of the inferior colliculus and nucleus reticularis pontis caudalis. In several experiments label was also apparent within the substantia nigra.

In one experiment, the injection invaded the mesencephalic nucleus V. Labeled axons could be traced into the motor nucleus V, the chief sensory nucleus V and into a region slightly medial or possibly within the descending nucleus V.

Finally, labeled axons pass to the contralateral colliculus via the tectal commissure. These axons appear to arise and end primarily within the deeper tectal layers.

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1548 PROJECTION FROM LATERAL GENICULATE ONTO VISUAL CORTEX IN ADULT CATS. A QUANTITATIVE ANALYSIS WITH HORSERADISH-PEROXIDASE. H. Holländer* and H. Vanegas. Max-Planck-Inst. Psychiat., Munich, and Inst. Venezolano Invest. Cientif., Caracas. (Partly supported by the A.v.Humboldt-Stiftung)

The location and size of the nerve cells which project from the dorsal lateral geniculate nucleus (LGNd) onto the visual cortex was investigated by means of single injections of horseradish-peroxidase (HRP) into area 17 (4 cases), area 18 (6 cases) and area 19 (1 case). Seventeen hours to 7 days after intracortical injection of 0.03-0.06µl of a 30% aqueous solution of HRP, the animals were perfused and the brains were stereotaxically blocked, sectioned transversely at 40µm and developed for HRP activity. The location and extent of every injection was determined with respect to the cytoarchitectonic subdivisions of the visual cortex. The location of every retrogradely-labelled nerve cell was plotted from each, unstained section, and the LGNd subdivisions were drawn in after counterstaining with thionin. The number of labelled nerve cells in each LGNd subdivision was estimated from these drawings. Subsequently, one case each for injections in areas 17, 18 and 19 was chosen for quantitative analysis, namely:

1. The number of labelled nerve cells in each LGNd subdivision was expressed as percent of the total number of labelled nerve cells in that particular case (Table 1). This shows that area 17 receives a considerable projection from laminae A and A1, a small one from lamina C and from laminae C1 to C3 taken as a whole, and none from the medial interlaminar nucleus (MIN). Area 18 receives a significant projection from lamina A1. The input from lamina A is meager, but that from lamina C is significant. Also significant is the input from MIN and, to some extent, laminae C1-3. Area 19 receives a considerable projection from MIN, a significant one from laminae C and C1-3, and none from lamina A. On the whole, the projection source shifts from the A-laminae to the C-laminae plus MIN as one goes from area 17 to 18 and to 19.

Table 1

Inj. area	% labelled nerve cells					
	A	A1	C	C1-3	MIN	other
17	34	50	5	3	0	8
18	1	26	26	11	25	11
19	0	2	14	19	40	25

2. The perikaryal size of every labelled nerve cell which showed a nucleolus (except those nerve cells near or at boundaries between LGNd subdivisions) was determined by planimetry. Data were similarly obtained from representative samples of unlabelled nerve cells located, for each case and each LGNd subdivision, within the region where the labelled nerve cells were found. Table 2 shows such values for LGNd subdivisions with a sufficiently large number (17 to 187) of labelled nerve cells.

Table 2

Inj. area	size of labelled/unlabelled nerve cells (mean in μm^2)				
	A	A1	C	C1-3	MIN
17	310/238	270/239	248/220	155/133	
18		592/299	476/222	294/153	446/296
19			520/386	261/232	367/317

After injection in either area 17 or 19, the labelled nerve cells in LGNd are 1.1-1.4 X larger than the unlabelled ones. After area 18 injection, however, the labelled nerve cells are 1.5 (MIN) to around 2 X (A1, C and C1-3) larger than the unlabelled ones. This indicates that area 18 receives its input almost exclusively from the largest neurons of any LGNd subdivision considered.

These results reveal a remarkable degree of especialization in the geniculo-cortical organization, and are relevant for the question of parallel-channel processing and the X-Y dichotomy in the visual system.

1549 TRANSNEURONAL TRANSPORT OF ^{3}H PROLINE WITHIN THE VISUAL SYSTEM OF THE GREY SQUIRREL. M.F. Huerta*, V.A. Casagrande, J.T. Weber and J.K. Harting. (SPON: A.W. Clark). Depts. of Anatomy, Univ. of Wisconsin, Madison, WI. 53706 and Vanderbilt Univ., Nashville, TN. 37202.

We have used the method of transsynaptic transport to autoradiographically analyze the organization of ocular inputs to the visual cortex of the grey squirrel. Following intraocular injections of 2.5 Mci of ^{3}H-proline, dense continuous bands of label could be seen filling layer IV of both the contralateral and ipsilateral striate cortex. We could find no evidence of ocular separation of inputs. Contralaterally the label could be seen forming a continuous band throughout striate cortex. This band was thickest laterally (near the representation of central vision), and thinnest medially, matching the width of layer IV as seen in the nissl stain. A similiar pattern was seen in the ipsilateral striate cortex, except that the label was restricted to the binocular region.

Our autoradiographs also revealed sparse label within area 18 bilaterally, as well as within a region of the contralateral temporal cortex which lies ventral, but not immediately adjacent to area 18.

Well known subcortical targets of the retina contained dense transported protein. These included bilateral projections to the suprachiasmatic nucleus of the hypothalamus, the dorsal and ventral lateral geniculate nuclei, the pretectal complex and the superficial layers of the contralateral superior colliculus. Label within the ipsilateral colliculus was limited to rostral levels (but not the most rostral tip) where it occurred in islands or patches which lay within the stratum opticum.

Dense label was also apparent within several subcortical regions which are not retinal targets. These include the pulvinar nucleus, the parabigeminal nucleus, the most lateral portion of the mesencephalic reticular formation and the pontine gray. The label within the pontine gray occured in discrete patches and was heaviest within the dorsal lateral region. All of these regions are known to receive input from the superficial layers of the colliculus.

Finally, several labeled axons could be identified crossing within the dorsal tegmental decussation to enter the predorsal bundle. Since this bundle arises exclusively from the deeper tectal layers it appears that the tritiated precursor is able to pass through at least two synapses.

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1550 LIGHT-INDUCED POTASSIUM FLUX AND ITS RELATIONSHIP TO GLIAL AND EXTRACELLULAR SLOW POTENTIALS IN THE RETINA OF NECTURUS

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Light-evoked changes in extracellular potassium concentration ($[K^+]_o$), intracellular glial responses, and extracellular slow potentials were recorded from the retina of the mudpuppy, Necturus maculosus. The potassium response consists of increases in $[K^+]_o$ in the proximal retina at both light onset and offset. In the distal retina, $[K^+]_o$ slowly decreases at light onset, and returns to the resting level at light offset, as previously reported at this meeting by Oakley and Green (1974). Maximum K-increase occurs at 20% retinal depth, and thus proximal to the current sink of the b-wave of the electroretinogram. However, graphs of the amplitudes of the K-increase and the proximal negative response (PNR) as a function of depth are nearly identical. Also, like the PNR, the K-increase exhibits similar dependencies on stimulus position and diameter. It thus seems probable that the K-increase is generated by the same neurons which generate the PNR.

The depolarizing response of Müller cells is generally similar to the K-increase in waveform and comparative behavior to a wide variety of stimulus conditions, except that the Müller cell response has a somewhat faster rise time. Thus, given that glial cells elsewhere in the nervous system are depolarized by increases in $[K^+]_o$, it is likely that the observed Müller cell depolarization is primarily a result of the proximal K-increase.

In eyecups drained of vitreous humor, the K-increase and Müller cell responses are larger than in eyecups where the vitreous is not removed. However, other properties of these responses are largely unaffected by the depth of the vitreous. A prominent, on/off, negative-going, field potential can be observed in the proximal retina of eyecups drained of vitreous. This response which we call the M-wave, exhibits a remarkable similarity to Müller cell responses in both waveform and comparative behavior. A small M-wave is likely present in eyecups where the vitreous has not been drained. It thus appears that the light-evoked K-increase in the proximal retina induces a Müller cell depolarization which in turn generates the M-wave. The relationship of these responses to the b-wave of the ERG remains unclear.

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1551 THE ROLE OF PRESTRIATE CORTEX IN THE RECOVERY OF VISION AFTER AREA 17 LESIONS. E. Gregory Keating. Depts. Anat. & Neuro., S.U.N.Y. - Upstate & V.A.H., Syracuse, N.Y. 13210

A number of visual skills survive complete removal of area 17 in the monkey. It is thought that this recovery of vision may be due to sparing of adjacent prestriate cortex since the prestriate cortex receives visual information via a tectal - pulvinar pathway. The result of this study is that at least certain abilities in the destriated monkey survive additional removal of prestriate areas OA, OB & most (perhaps all) of TEO.

Five rhesus were trained on several tasks. They then received bilateral complete area 17 lesions. If, by behavioral criteria, the lesions were judged complete the monkeys were retrained on, 1) a color discrimination, 2) a measure of their accuracy in reaching toward lighted targets, 3) absolute and relative velocity discriminations. The hue discrimination task was designed to be definitively free of flux cues regardless of the animal's spectral sensitivity (Kicliter & Loop, Vis. Res. , 1976). Prestriate cortex was then removed in 4 of the monkeys and they were again retested. The striate lesions have been verified histologically in 3 animals. The results confirm that completely destriated monkeys can relearn all of these tasks.

However, these recovered abilities to do not depend heavily on the integrity of the remaining occipital areas. In 3 monkeys prestriate lesions disrupted performance but did not prevent relearning of any of the tests. The prestriate lesion, confirmed in 2 monkeys, included all of OA, OB & most of TEO. In both animals there remained a spared group of cells in the tectorecipient portion of the inferior pulvinar nucleus. In a third monkey, sill alive, the prestriate lesion was extended more anteriorly into the temporal lobe to include the entire cortical target of the inf. pulvinar (Benevento & Rezak, Br. Res., 1976). This animal has also relearned all of the behavioral tests. Thus, retinal projections to the midbrain appear sufficient for these visual capabilities. The rest of the extrastriate pathway from tectum to pulvinar to cortex is not critical for the kinds of vision measured by these tasks. (Supported by NS10576 & V.A. - M.R.I.S. 4847-01)

1552 MULLER-LYER ILLUSION IN DISCONNECTED RIGHT AND LEFT CEREBRAL HEMISPHERES.
Santosh Kumar, Eran Zaidel* and Joseph E. Bogen. California Institute of Technology, Pasadena, CA 91109 and Ross-Loos Medical Group, Los Angeles.

We previously suggested (Kumar and Bogen, 1974) that the Muller-Lyer Illusion has lateralizing significance on the basis of findings in patients with lateralized lesions using an apparatus designed by Jalota (1963) to apply the psychophysical method of adjustment. This method, in which the stimulus is exposed for a long period, has been adapted for use with commissurotomy patients, whose visual testing has usually involved tachistoscopic brief exposure to either the right or left visual field in order to lateralize the sensory input to one or the other hemisphere.

In order to confirm in the split-brain the findings with lateralized lesions, use has been made of the technique devised and developed by Zaidel (1974) to lateralize complex stimuli needing longer exposure time. A contact lens on which is mounted a tiny collimator permits occlusion of one visual half-field for an extended period.

The method of adjustment could thus be used to study the Muller-Lyer Illusion in the disconnected hemispheres of two patients, one female (NG) and one male (LB). The Muller-Lyer Figure was presented both horizontally and vertically. Adjustment was done with inward and outward (or upward and downward) movement to left or right of (or above or below) the standard (constant) stimulus.

The basic ABBA order of stimulus presentation was followed to counterbalance the effect of practice and fatigue, if any, and testing was done in two sessions on different days for each patient. Both visual fields were tested in each session. The order of presentation was completely reversed the second day.

80 Observations were made on each hemisphere. In the horizontal presentation, the illusion effect was 12.5% for the left hemisphere and 45.2% for the right hemisphere of NG; it was 7.6% for the left hemisphere and 35.2% for the right hemisphere of LB.

In the vertical presentation, the illusion effect was 15.4% for the left hemisphere and 32.% for the right hemisphere of NG; it was 9.7% for the left hemisphere and 27.9% for the right hemisphere of LB.

Differences between left and right hemispheres of either patient in either the horizontal or vertical condition were significant beyond the 0.001 level of confidence (2-tailed t-test for difference between means with unequal variances).

There was no significant difference in whether the variable was to the right or left of the standard stimulus; or above or below it. There was little difference between the inward or outward movements of the variable stimulus. This applied both for the horizontal and vertical presentation.

In conclusion, the horizontal presentation differentiated much better between the hemispheres than did the vertical presentation. The results clearly show that the right hemisphere saw a greater Muller-Lyer Illusion than did the left hemisphere.

Jalota, S. A students' manual of experiments in psychology:qualitative and quantitative. Bombay, India: Asia Publishing House. 1963.

Kumar, S. and Bogen, J.E. Does the Muller-Lyer Illusion have lateralizing significance? Abstr. Soc. Neurosc. Fourth Annual Meeting 1974.

Zaidel, E. A technique for presenting lateralized visual input with prolonged exposure. Vision Res. 15:283-289, 1974.

1553 GABA MEDIATED NEURONAL MECHANISMS IN THE MUDPUPPY RETINA. Robert F. Miller and Ramon Dacheux.* Neurosensory Laboratory, Dept. Physiol., SUNYAB, Buffalo, NY 14214.

A number of uptake and release studies have implicated GABA as a possible neurotransmitter in the vertebrate retina. We have studied the effects of GABA and the GABA antagonist picrotoxin on different neurons in the perfused retina-eyecup preparation of the mudpuppy. Effects of 1mM GABA were evaluated in either of two conditions. In one set of experiments GABA was added to the perfusate, while in a second series GABA was added after synaptic transmission was blocked by a perfusate containing 2mM cobalt. In some cells GABA effects were evaluated before and after cobalt treatment. A bridge device was used to monitor input resistance. Receptors and the majority of horizontal cells were relatively insensitive to GABA application. A small percentage of "horizontal cell like" units were depolarized by GABA. This depolarization is chloride dependent and the chloride equilibrium potential of these cells is more + than the dark membrane potential as indicated by studies using chloride specific microelectrodes. Bipolar cell responses were reduced in amplitude by GABA, an effect largely mediated through a decrease in input resistance, since the membrane potential was only minimally perturbed. This action of GABA is a direct effect on the bipolar cells since GABA application after treatment with cobalt also causes a decrease in input resistance. The effects of GABA appear to be more evident on the depolarizing bipolar cells compared to hyperpolarizing bipolars. On-off amacrine cells usually show a selective loss of the on response, and a reduction, but persistence of the off response. It is argued that this effect is largely a reflection of GABA action on bipolar cells, since the input resistance of amacrines is minimally changed by GABA.

The effects of picrotoxin (10^{-5}M) are opposite to those of GABA. Bipolar cell responses are enhanced and the input resistance is increased. This enhancement is largely secondary to a change in input resistance since the membrane potential is minimally effected by picrotoxin. An increase in amacrine cell responses by picrotoxin is attributed to the effects on bipolar cells. Picrotoxin also blocks some of the IPSPs observed in on-off ganglion cells. These latter cells are rapidly hyperpolarized by externally applied GABA. Intracellular measurements of chloride activity in these cells indicates that the chloride equilibrium potential is more negative than the resting membrane potential. These findings suggest that GABA receptors are clearly present on bipolar cells, on-off ganglion cells, and one type of horizontal-cell like neuron. The action of picrotoxin on bipolar cells raises the possibility that these neurons are being affected by GABA released in the dark, and that the action of GABA is to maintain the membrane potential of bipolar cells close to that determined by the action of receptor released transmitter.

1554 ORIENTATION SELECTIVE INHIBITION FROM BEYOND THE CLASSIC CORTICAL RECEPTIVE FIELD. J. I. Nelson and Barrie Frost. Dept. Physiol., JCSMR, ANU, Canberra, Australia 2601.

Recording from single units in area 17 of the cat, we have found strong, reliable orientation specific inhibition with a number of properties which make it unlikely to originate from the cortical receptive field as normally defined. We call this effect domain inhibition.

Orientation tuning curves for this domain inhibition effect were produced by systemmatic variation of the angle of a grating pattern adapting field. The contour adapting field was swept back and forth across an annular region (outer diameter 18.5 or 37°; inner diameter 5 or 10°) surrounding, but remote from, the receptive field of the cell under test. At the same time, the central region was stimulated optimally by a small, central bar sweeping back and forth, which we term a "jiggle" stimulus. The adapting field inhibited the elevated discharge elicited by the jiggling bar in most simple and hypercomplex type I units tested. Maximum inhibition was of the order of 50%, and was typically obtained when the grating moved with the sweep direction and bar orientation preferred by the unit. The conventional receptive field inhibitory sidebands show neither this direction nor orientation specificity.

Inhibitory sidebands are also highly localized. Using coarse 25% duty cycle gratings we were unable to reveal spatial structure in the region giving rise to domain inhibition. We have in one case also observed orientation domain inhibition to be much more velocity-selective than the cell's discharge center itself.

We attempted a direct test of the extra-receptive-field origins of domain inhibition on some units held for 15 to 26 hours. The extent of end-zone inhibitory regions (end-stopped inhibition) in hypercomplex type I cells was delimited by length-response curves. Inhibitory sidebands were revealed by PSTHs taken in the presence of a jiggle stimulus at 90° to the preferred orientation, and in the optimal orientation and sweep axis at a variety of positions above and below the receptive field center. From this data the receptive field boundaries were estimated by conventional means, and by computing both running t-tests and binomial probability functions. All methods indicated that the grating which produced orientation-specific inhibition was located outside the conventional limits of inhibitory end-zones and sidebands.

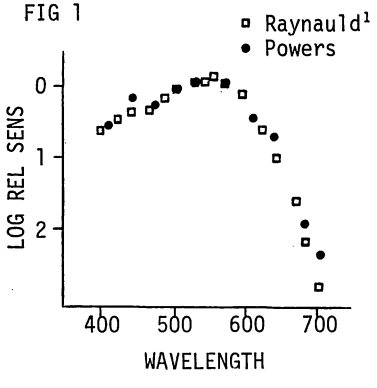
A substantial psychophysical literature indicates that a field of like-oriented contours can exert a strong influence on the perceived orientation of lines. We have evaluated domain inhibition as a possible physiological mechanism for orientation contrast by preparing a series of orientation tuning curves taken in the presence of adapting fields of differing orientations. Tuning curves are greatly affected in height by the adapting field. There is no shift in optimal orientation, although maximally-inhibitory orientations of the adapting field can reliably produce a flattening in the peak of the cell's orientation tuning curve. We conclude that orientation channels are not independent, and that the domain inhibition we have observed could produce the sensory coding errors seen in orientation illusions.

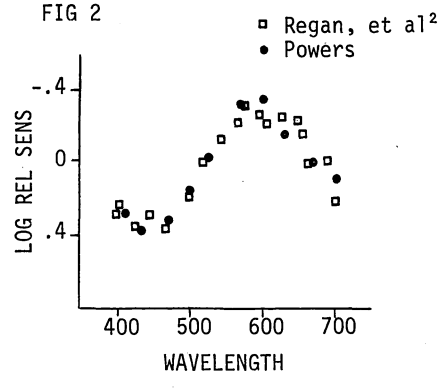
1555 BEHAVIORALLY MEASURED VISUAL SPECTRAL SENSITIVITIES OF THE GOLDFISH MATCH PHYSIOLOGICAL PREDICTIONS. Maureen K. Powers. Dept. of Psychol. and Div. Biol. Sci., Univ. of Mich., Ann Arbor, MI 48109.

We have obtained dark- and light-adapted spectral sensitivities for the goldfish, using a different behavioral technique for each. Our principal finding is that in both cases our results closely match those of electrophysiological workers.

DARK-ADAPTED SPECTRAL SENSITIVITY was obtained using classical conditioning of respiration rate. Absolute threshold was found for 3 fish at 10 wavelengths, and a spectral sensitivity was plotted from the results (Fig. 1). Our data agree very well with those of Raynauld[1], who recorded from single ganglion cells in the isolated retina of the goldfish. Interestingly, both show greater sensitivity in the red region than would be predicted if only rods were active. Neither absorption by the lens nor high optical density of porphyropsin can account for the shape of the curve. The possibility that the retina was not fully dark adapted was ruled out by histological examination of the retinomotor state, which showed that the cone myoids are extended and the pigment epithelium expanded. The possibility that our measurements were not made at absolute threshold for the rods is excluded by the very low ratio (about $1:10^3$) of effective quanta per rod. Our results suggest that (1) long wavelength sensitive cones determine absolute threshold at some wavelengths, and (2) even when the morphology of cones and pigment epithelium indicates that the retina is photomechanically dark adapted, the cones are not totally insensitive to light. The conclusion that two spectral mechanisms operate at absolute threshold raises the question of whether the dark adapted goldfish can make wavelength discriminations scotopically, and experiments are now in progress to test this possibility.

LIGHT-ADAPTED SPECTRAL SENSITIVITY was obtained using the dorsal light tilt reaction (the innate tendency of the fish to tilt its dorsal surface toward bright light). The fish was placed between two extended monochromatic fields, and the intensity of one of the fields was varied to find the value at which the fish did not tilt. A spectral sensitivity was constructed from these measurements for 4 fish (Fig. 2). Our data agree well with those of Regan, et al[2], who recorded both ERG and tectal evoked response in the intact goldfish. We find it interesting that the dorsal light reflex matches the electrophysiological results, and we suggest that for this reason it should be a behavior of choice for psychophysical experiments in these animals. (Supported by EY-00168 to S. S. Easter, Jr.)





1. Raynauld, Science 177:84, 1972.
2. Regan, Schellart, Spekreijse & Van den Berg, Vision Res. 15:799, 1975.

1556 NEURONAL ACTIVITY IN THE BRAINSTEM RETICULAR FORMATION DURING PERFORMANCE OF A "GO"-"NO GO" VISUAL ATTENTION TASK IN THE MONKEY. Eva Bakay Pragay* and Allan F. Mirsky, Sch. Med., Boston Univ., Boston, MA. 02118

Results obtained by macro-electrode recording and stimulation in the brainstem reticular formation (RF) suggest that this system is involved in maintaining performance in a successive visual discrimination ("attention") task. The present study was designed to substantiate this assumption at the neuronal level. Monkeys were trained to depress a hold key for a period of 2 sec. After this hold period, either a red or a green cue lamp appeared in a random sequence. The red lamp required the monkey to lift his hand from the hold key and hit a target button within 1 sec of the cue presentation ("go" trial). The green lamp required continued depression of the hold key for 1 sec following the presentation of the cue ("no go" trial). Correct performance was reinforced with fruit juice. Unit activity primarily was recorded in three subjects from the mesopontine RF and adjacent structures, as well as from the somatosensory cortex. A total of 194 task-related (136 subcortical and 58 cortical) units were studied. In the RF, a substantial number (35%) of the units showed changes in firing rate early in the trial, presumably reflecting stimulus onset and/or the "lift" response. In the majority (71%) of the cases, changes related to "go" trials were paralleled by changes related to no go trials. At the same time, in 48% of the cases, changes accompanying "no go" trials were of shorter duration and/or smaller magnitude (firing rate) than those with "go" trials.

Neuronal activity in the RF did not differ greatly from that observed in adjacent subcortical structures. However RF activity differed markedly from that in the cortex; these cells showed less (24%) "early" within trial activity and considerably less change in relation to no go trials.

The substantial amount of early change in the RF as well as the differential responsiveness to stimuli in go and no go trials support the view that this system is involved in information processing supporting visual discrimination. (Work supported by NIMH grants 12568 and K5-14915).

1557 PROJECTIONS FROM LAYERS V AND VI OF THE NEOCORTEX TO THE TECTUM AND THE THALAMUS OF GALAGO SENEGALENSIS.
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The key to understanding the laminar organization of the cortex lies in determining the connections of its various layers; the retrograde transport of horseradish peroxidase provides a unique opportunity to study the efferent projections from the various layers. In the present experiments we have continued our investigation of the sensory pathways of Galago senagalensis by injecting horseradish peroxidase into the tectum and thalamus and identifying labeled cells in the cortex as well as the brainstem centers of the ascending pathways. The chief results show that cells of layer V of the cortex project to the tectum and cells of layer VI project to the thalamus. The projections from layer V to the tectum are precisely organized according to the various cortical cytoarchitectonic subdivisions. After injections confined to the superficial superior colliculus only area 17 showed labeled cells. After injections of the deep layers of the superior colliculus an extensive cortical area, intercalated between the koniocortex of the occipital and temporal lobes, shows labeled cells. Thus, the evidence suggests that much if not all of the belt cortex surrounding the primary visual, auditory, and somatic areas projectsto the deeper layers of the superior colliculus.

The projections from layer VI to the thalamus are also organized according to cytoarchitectonic subdivisions of the cortex. When the superior pulvinar is injected an extensive zone of the temporal cortex shows labeled cells below, behind, and in front of the middle temporal area. When the tecto-recipient zone of the pulvinar (the caudal portion of the inferior pulvinar) is injected, the middle temporal cortex is also labeled. Finally, injections of the dorsal lateral geniculate lead to labeled cells in area 17. Thus, it appears that layer VI is reciprocating the projection from the thalamus to layer IV.

These experiments, in addition, cast light on the ascending connections to the tectum. Injections of the deeper layers of the superior colliculus resulted in labeled cells in the lateral tegmental area and the inferior colliculus suggesting a source or sources for auditory input to this center. Injections of the superficial layers of the superior colliculus led to labeled cells in the parabigeminal nucleus. Finally, the question: "Are there truly intrinsic subdivisions of the pulvinar?" can also be addressed by these methods. As expected, injections of the tecto-recipient zone of the pulvinar (as defined by anterograde degeneration) led to labeled cells in the superior colliculus (superficial layers). Injections of the non-tecto-recipient zone of the pulvinar did NOT uncover any major source of visual impulses from the brainstem. For example, only a few cells in the pretectum were labeled whatever portion of the pulvinar was injected. Indeed, comparing the size of the pretectum and pulvinar in primates and other mammals suggests that the pretectum did not expand in proportion to the expansion of the pulvinar. On the contrary, the data suggest that other thalamic nuclei--midline and interlaminar nuclei--may project to the superior pulvinar. Thus, this subdivision may be intrinsic as this term was first used by Rose and Woolsey (EEG Clin. Neurophysiol., 1949, 1:391-404). (Supported by NIMH Grant MH-4849.)

1558 GENESIS OF CONNECTIONS IN THE VISUAL SYSTEM OF FETAL MONKEY BRAIN. Pasko Rakic, Dept. of Neuropathology, Harvard Med. Sch. and Dept. of Neuroscience, Children's Hospital Medical Center, Boston, Mass. 02115.

The prenatal development and pattern of termination of neuronal connections subserving ocular dominance in the dorsal lateral geniculate body (LGd), superior colliculus (SC) and primary visual cortex (area 17) of the rhesus monkey were analyzed using anterograde axoplasmic and transneuronal transport of tritium labeled tracers. Equal amounts of H^3-proline and H^3-fucose (total 1.0 - 1.5 mCi) were injected into the vitreous body of one eye of monkey fetuses of different gestational ages. (Gestation in the rhesus monkey lasts 165 days.) Following injection, the fetuses were returned to the uterus and 14 days later again removed from the uterus by a second caesarian section, fixed with mixed aldehydes by intracardiac perfusion and their brains processed for autoradiographic analysis.

In the youngest fetus of the series, that was injected at embroynic day 64 (E64) and killed at E78, the label transported in the axons of retinal ganglion cells was uniformly distributed throughout the full extent of both LGd's (which at this age have their full complement of neurons but no laminae) and SC's on both sides. Radioactive tracer transferred transneuronally to principal neurons of LGd appears in the geniculocortical fibers which at this fetal age form the optic radiation in the cerebral wall of the occipital lobe, but the great majority of these axons do not seem to enter the cortical plate. Instead, axons accumulate in the intermediate zone below the developing cortex. At this fetal age, as demonstrated by H^3-thymidine autoradiography (Rakic, P., 1974, Science, 183:245), the majority of neurons of layer IV, which will eventually receive input from geniculocortical axons, are not yet generated and many of those that have been generated are in the process of migration.

In the fetus injected at E110 and killed at E124, label in the LGd is distributed selectively in the territory occupied by the emerging laminae 1, 4 and 6 contralateral and 2, 3 and 5 ipsilateral to the injection site. In the SC of this fetus, the grains are distributed in a pattern of alternating dense and light concentrations, indicating segregation of input from the two eyes. By this fetal age all cortical neurons have been generated and have completed their migration to the cortex. Numerous geniculocortical fibers pass from the optic radiation towards area 17 and a substantial number of these fibers enter the developing cortex and their endings are uniformly distributed in layers IV and VI.

In the oldest fetus of the series, that was injected at E130 and killed at E144, distribution of the label in the LGd and SC has virtually attained the adult pattern. Transneuronally transferred radioactivity in layer IV of the primary visual cortex of this fetus is by now segregated into the sublayers IVA and IVC. In addition, counts of grains overlying this layer demonstrate alternating low and high concentrations that indicate emerging ocular dominance columns.

In summary, it appears that in fetal monkey brain neuronal projections carrying input from the two eyes initially overlap; they segregate during the second half of gestation and become fully separated in the LGd and SC and partially separated in the visual cortex by at least three weeks before birth. (Supported by NIH Grant NS 11233.)

1559 TOPOGRAPHICAL ORGANIZATION OF EXTRAGENICULATE THALAMIC PROJECTIONS TO STRIATE CORTEX AND PRESTRIATE CORTEX IN THE MACAQUE MONKEY. Michael Rezak and L. A. Benevento. College of Medicine, University of Illinois at the Medical Center, Chicago, Illinois 60680.

Our previous autoradiographic and degeneration studies (e.g., Brain Res. '75; '76) have shown that the inferior pulvinar (PI) and adjacent lateral pulvinar project to layer I of striate cortex (area 17) and to layers IV-III and I of prestriate cortex. These studies have also given anatomical evidence for the organization of the representation of the visual hemifield in PI. It was of interest to extend these studies in order to determine whether the topography of the extrageniculate projections to layer I of striate cortex is organized according to the representation of the visual hemifield as are the geniculate projections to layer IV of striate cortex. The present studies were carried out with cortical microinjections (0.15 μl) of horseradish peroxidase for retrograde labelling of thalamic cells and thalamic microinjections (0.15 μl) of tritiated proline and leucine for anterograde labelling of thalamocortical axons. A total of 15 rhesus monkeys (Macaca mulatta) were used. In the autoradiographic studies there was no leakage in the cortex and in each case the injection was confined to the thalamus. All thalamic injections confined to PI resulted in label which was distributed in layer I of striate cortex. When the injection site was located in the dorsal portion of PI (i.e., lower visual quadrant) label was found in dorsomedial and dorsolateral striate cortex which also represents the lower visual quadrant. When the injection site was located in the ventral portion of PI (i.e., upper visual quadrant) label was found in ventrolateral and ventromedial striate cortex which also represents the upper visual quadrant. When the injection sites were located along the horizontal meridian in PI the label was found in the middle of lateral striate cortex, i.e., along the representation of the horizontal meridian. In these same cases, each single injection produced, in addition to the label in layer I of area 17, a distribution of label in prestriate cortex which was not confined to a particular region, but instead, formed several distinct "patches". The location of the patches in prestriate cortex corresponded to the location of the label in layer I of striate cortex with reference to the representation of the visual hemifield. For example, a single injection in the portion of PI which represents the upper visual quadrant produced label in layer I of ventrolateral striate cortex and several patches of label in ventrolateral prestriate cortex. These studies indicate that the projections of PI to layer I of striate cortex are organized according to the topographical representation of the visual hemifield and that the projections of PI to layers IV and III of several regions of prestriate cortex are arranged in the same fashion. Cortical injections of horseradish peroxidase support the autoradiographic findings. For example, an injection in dorsolateral striate cortex resulted in retrograde labelling of cells in dorsal PI while an injection in ventrolateral striate cortex resulted in retrograde labelling of cells in ventral PI. Injections of dorsolateral and ventrolateral prestriate cortex also produced labelling in dorsal and ventral PI respectively. Interestingly, simultaneous injections, in the same hemisphere, of both striate and prestriate cortices which represent the same portion of the visual hemifield produced retrograde labelling of approximately the same number of cells as would either one of the injections alone. This result might indicate that some of the sustaining projections to striate cortex and several regions of prestriate cortex from PI are made up of axon collaterals rather than axons of individual neurons, each of which represent the same point in the visual hemifield.

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1560 PROPERTIES OF GANGLION CELLS IN THE RETINA OF THE BRUSH-TAILED POSSUM, TRICHOSURUS VULPECULA. M.H. Rowe, E. Tancred*, B. Freeman* and J. Stone*
Sch. Anat., U. New South Wales, Sydney, Australia.

As a marsupial, the brush-tailed possum provides an opportunity to determine which of the many features of ganglion cell organisation found in the domestic cat may be widely distributed among mammals. An isodensity map of whole-mounted possum retina shows a concentration of ganglion cells in the superior temporal quadrant, about 3mm from the optic disc, which corresponds to the area centralis of the cat. There is also a marked elongation of the isodensity lines along the naso-temporal axis forming a clear visual streak just above and parallel to the lower border of the tapetum.

Field potentials elicited by optic chiasm stimulation and recorded at the optic disc consist of 3 prominent components suggesting the existence of at least three conduction velocity groupings among optic nerve fibers. These groups have been labelled t_1, t_2, and t_3 following previous work on cat optic nerve. Recordings from around the perimeter of the disc suggest that the ganglion cells giving rise to t_1 and t_3 fibers are more or less evenly distributed among all retinal quadrants. The t_2 component, however, is most prominent in the superior temporal quadrant, and is almost absent on the nasal side of the disc, suggesting that the cells giving rise to these fibers are most concentrated in the superior-temporal quadrant.

Single unit recordings indicate that the cells whose axons comprise the t_1 conduction velocity group have receptive field properties similar to those observed in Y-type ganglion cells of the cat retina. The cells giving rise to t_2 and t_3 fiber groups differ from t_1 cells in having very tonic responses to stationary visual stimuli, but there appears to be no sharp distinction between the receptive field properties of t_2 and t_3 cells.

A few units have been encountered whose latencies are too long to be included among the t_3 group, suggesting the existence of a fourth group of cells. So far, however, the sample is too small to determine their relative frequency or the variety of their receptive field properties.

1561 PROJECTIONS OF THE SUPERIOR COLLICULUS TO THE INTRALAMINAR THALAMIC NUCLEI IN THE CAT AND RHESUS MONKEY. G.J. Royce, N. L. Strominger, and J.K. Harting. Department of Anatomy, University of Wisconsin, Madison, WI 53706.

Autoradiographic and horseradish peroxidase tracing methods have been used to study the projection of the superior colliculus upon the intralaminar thalamic complex in cats and rhesus monkeys.

Following injections of ^{3}H proline into the deeper tectal layers of the cat there is extensive transported radioactive protein within the caudal and rostral divisions of the intralaminar complex. Within the caudal division, the parafascicular nucleus contains dense label, both medial and lateral to the fasciculus retroflexus. Less dense label is present within the adjacent centromedian nucleus, and is interpreted as being predominantly fibers of passage. At rostral levels of the intralaminar complex dense label is present within the paracentral and central lateral nuclei, while only sparse label is apparent within the central medial nucleus. In several cats, horseradish peroxidase was injected into both rostral and caudal divisions of the intralaminar complex and labelled neurons were observed only within the deeper layers of the colliculus.

Following injections of tritiated proline into the deeper layers of the monkey superior colliculus, the pattern of transported protein within the caudal intralaminar complex is similar to that described for the cat. Since the centromedian nucleus is much larger in the monkey, it is easier to establish that the tectal input terminates within only a very restricted medial portion of the nucleus, if at all. In contrast to the sparse label within the centromedian nucleus, transported protein within the parafascicular nucleus is quite dense and forms a dorsal "cap" which surrounds the fasciculus retroflexus. Within the rostral divisions of the monkey intralaminar complex there is considerable transported protein within the paracentral, central lateral and central inferior nuclei. The projection to the paracentral nucleus is strikingly bilaminar, consisting of a dense medial lamina which invades the adjacent dorsomedian nucleus and a thinner, lateral band within the dorsal lateral portions of the paracentral nucleus.

Finally, our autoradiographs reveal that, in the monkey, the projection of the superior colliculus upon the intralaminar complex arises exclusively from the deeper tectal layers. No transported protein was observed within any region of the intralaminar complex following injections restricted solely to the superficial tectal layers.

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1562 AN AUTORADIOGRAPHIC STUDY OF THE PROJECTIONS OF THE PRETECTUM IN THE MACAQUE MONKEY. Rebecca Santos-Anderson, Michael Rezak and L. A. Benevento. College of Medicine, University of Illinois at the Medical Center, Chicago, Illinois 60680.

Prior to studying the projections of the pretectum of the rhesus monkey (Macaca mulatta) with the autoradiographic technique, we determined which subdivisions of the pretectum receive projections from the retina, retinorecipient superficial layers of the superior colliculus (SC), and the occipital cortex. These studies were done by intraocular injection of tritiated fucose and proline, microinjection of tritiated amino acids and the Fink-Heimer method, respectively. Using another group of 7 monkeys a single unilateral 0.3 µl injection of tritiated leucine and proline was made in the pretectum of each animal. Following injections of the sublentiform nucleus, which receives input from the retina, superficial layers of the superior colliculus and occipital cortex grains were found bilaterally in the nucleus of the pretectal area (NPA), the nucleus of the posterior commissure (NPC), all components of the Edinger-Westphal or visceral oculomotor nuclei and contralaterally in the opposite sublentiform nucleus (SL). Ipsilaterally, grains were found in the deep layers of SC, the mesencephalic reticular substance (MRS) about the red nucleus, the interstitial nucleus of Cajal (INC), the nucleus of Darkschewitch (ND), as well as the olivary nucleus, limitans nucleus, zona incerta, pregeniculate nucleus, peripeduncular nucleus, anterior tegmental nucleus, thalamic reticular nucleus, subfascicular nucleus (Sf), reunions nucleus (Re), intralaminar nuclei (IL) and the hypothalamus. Injections involving the olivary nucleus which also receives input from the superficial layers of the superior colliculus, retina and occipital cortex, resulted in a heavy concentration of grains in the contralateral lateral visceral nucleus of the Edinger-Westphal complex.

Following injections of NPC, which does not receive input from the retina, superficial superior colliculus and occipital cortex, grains were found bilaterally in the deep layers of SC, MRS about the red nucleus, pontine reticular substance and pontine nuclei. Contralaterally, grains were found in NPA and NPC. Ipsilaterally, grains were found in the central gray, SL, ND, INC, Sf, IL, Re and hypothalamus.

Grains were found at the border of the lateral pulvinar and medial pulvinar and in the oral pulvinar only when the injection was in the dorsal posterior portion of the pretectum and involved the deep layers of the superior colliculus. Our recent autoradiographic data show that these regions of the pulvinar project to area 7.

These results indicate that the retina, superior colliculus and occipital cortex can only influence the visceral nuclei of the oculomotor complex, and hence the intrinsic musculature of the eye, by virtue of their projections to the sublentiform and olivary nuclei of the pretectum. Pretectal zones which do not receive these various visual inputs, e.g., NPC, tend to project posteriorly and do not have direct connections with the Edinger-Westphal visceral complex. These studies also indicate that there might not be direct projections from the retino-recipient portion of the pretectum to dorsal thalamic nuclei which project, in turn, to visual cortices as has been demonstrated in the opossum and cat.

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1563 EFFECT OF MONOCULAR DEPRIVATION ON VISUAL BEHAVIOR IN RATS. Michael L. Schwartz and Lawrence A. Rothblat. Dept. Psychol., George Washington Univ., Washington, D.C. 20052

Monocular deprivation in the rat produces a severe loss of visually responsive neurons in the deprived cortex (Shaw, et al, Exp. Neurol. 45: 42, 1974). In addition, pyramidal cells of layer V show a reduction in spines on their apical dendrites (Fifkova, Comp. Neur. 140: 431, 1970). The present experiment examined the consequences of monocular deprivation on visual behavior.

Twelve hooded rats were reared from birth in a light proof room. At approximately thirty days of age all animals underwent unilateral lid suture, after which they were individually caged in a room with a twelve hour light-dark cycle and allowed monocular visual experience for 45 days. At the end of this time period all animals were trained to perform a simple flux discrimination in an automated apparatus. Following criterion performance on the flux task, the deprived eye of half the animals was opened and the experienced eye was closed (cross-sutured). For the rest of the animals the experienced eye remained open. All subjects were then trained to discriminate between columns and rows of 1/4 in. squares which were equated for flux. Thirty trials were given each day until the subjects reached a criterion of 90% correct in a daily session or received a total of 1200 trials. Whereas subjects with the experienced eye learned the pattern discrimination in a mean of 520 trials, the cross-sutured animals required a mean of 1095. In fact, 4 of 6 cross-sutured subjects failed to acquire the discrimination in 1200 trials. A subsequent test revealed that these animals were able to reacquire the original flux discrimination using the deprived eye. The impairment in form perception reported here is similar to the behavioral effects of monocular deprivation previously found in the cat (Ganz and Haffner, Exp. Brain Res. 20:67, 1974). However, since the rat has almost complete decussation of optic nerve fibers, the present deprivation effects are difficult to account for in terms of binocular competition (Wiesel and Hubel, J. Neurophysiol. 28: 1029, 1965). (Supported by NIH Biomedical Support Funds granted by the University Committee on Research, George Washington Univ.)

1564 NEURAL MECHANISMS MEDIATING PATTERN AND FORM DISCRIMINATIONS IN THE CAT. J.Sprague, M.Berkley, J.Tunkl* and G.Berlucchi*. Depts. Anat., Univ.Pa., Phila., Pa. 19174, Psychol., Fla.State U., Tallahassee Fla., 32306, Physiol., Univ. Pisa, Italy.

Thirteen representations of the visual field have been identified in cat cortex by unit recordings (Tusa et al, Soc. Neurosci. 1975). These receive thalamic input from 3 channels in lateral geniculate (A-A_1, C-C_1-C_2, NIM) (Rosenquist, Edwards & Palmer, Brain Res.80:71, 1974; LeVay & Gilbert, Brain Res., in press) and at least 3 divisions of pulvinar complex (Rosenquist et al, Soc. Neurosci. 1975 & unpublished). Performance in visuomotor behavior (attention, orientation, following, spatial localization, placing, perimetry) and in flux (dark-light), pattern (vertical-horizontal gratings), and form discriminations (differing in orientation and shape) was studied before and after removal of different sectors of these cortical fields. Discrimination was tested in 2-choice apparatus, with discriminanda transilluminated with at least 10X contrast. Errors were scored when negative, locked door was pushed. Learning and retention were evaluated by fixed criterion (90% or better correct on 2 successive days) and by means of a significant run to 0.05 and 0.01 levels of confidence. Removal of areas 17-18 (with retrograde atrophy of LGN_d laminae A-A_1) resulted in minimal or no deficits in visuomotor tests, or in retention of flux, pattern and form discrimination including an embedded figure, figure-ground reversals and cue reversals. Postoperative learning was within normal range for gratings and most form discriminations, but one discrimination differing in orientation was prolonged. Learning of flux was shortened. Removal of middle and posterior suprasylvian gyri, including parts or all of areas 19,20,21,LSA (with retrograde atrophy in lateral, inferior and medial pulvinar) and sparing much of 17-18, resulted in deficits in following and gross depth perception, and in retention and learning of form discriminations differing in orientation or shape. Flux discriminations were learned within normal range and gratings were only slightly prolonged. Smaller lesions involving area 19,21 and LSA resulted in similar deficits; lesions limited to area 20 caused marked deficits in original learning but none in retention. Other cats were trained in a different apparatus involving 2-choice flux, pattern and form discriminations presented at a fixed distance (36 cm); the forms used were small, thin outline figures subtending a visual angle of ca. 7.5°. After removal of areas 17-18, learning to 80-85% criterion was achieved but prolonged and performance was unstable. When required to discriminate between circles and multisided figures (7.5° VA at 36 cm) the threshold for normal cats was a 10-12 sided figure, and for cats lacking areas 17-18 was a 5-6 sided figure. We conclude that in this species areas 17-18 are neither necessary nor sufficient for learning or retention of flux and simple, large planimetric pattern and form discriminations or embedded figures. These functions are mediated primarily by cortex in suprasylvian gyri and sulci. Cats with 17-18 lesions have difficulty with pattern and form discriminations if the discriminanda are near the acuity threshold in size or in differences in shape. (Supported by EY-00577 and EY-00953).

1565 SENSITIVITY, ADAPTATION AND FUNCTION OF R7, AN ULTRAVIOLET RECEPTOR, IN DROSOPHILA. William S. Stark and Karin G. Hu, Department of Psychology, The Johns Hopkins University, Baltimore, Md. 21218.

The Drosophila retina has 8 receptors per ommatidium of three anatomically distinct types with different spectral sensitivities: R1-6, R7 and R8. (Harris, Stark and Walker, J. Physiol. 256: 415, 1976). Interest has recently focused on R7 which has been shown to be an ultraviolet (UV) receptor, a finding unexpected from previous data (Eckert, Kybernetik 9: 145, 1971). R7 was shown to have a UV rhodopsin (R) photointerconvertible with a stable 470 nm metarhodopsin (M). Studies of the electoretinogram (ERG) and phototaxis of normal Drosophila and mutants missing R1-6 and/or R7 (characterized by Harris, Stark and Walker) were used to determine the sensitivity, adaptation and behavioral function of R7. The ERG sensitivity of R7 in an R1-6 degeneration mutant increases relative to R8 in the first week of adult life allowing estimation of the spectral sensitivity of R7 alone; R7 is a one-peaked UV receptor which is 3 - 3.5 log units more sensitive at 350 nm than at 600 nm. Bright adaptation with 370 nm inactivates R7 and generates an ERG negative afterpotential. Bright adaptation with 470 nm reactivates R7 and repolarizes the ERG. Thus, maximal R to M conversion probably inactivates R7 through generation of a prolonged depolarizing afterpotential as was shown for R1-6 (Minke, Wu and Pak, J. comp. Physiol., 98: 345, 1975). Dark adaptation after bright adaptation with 470 nm asymptotes in about 1 min; after bright adaptation with 370 followed by 470 nm it asymptotes to about the same threshold in 5 min showing a neural component of adaptation not reflected in the immediate ERG repolarization and M to R reconversion. One generation of vitamin A deprivation decreases R7 sensitivity by about 2 log units but does not change the spectral sensitivity shape. By comparison, vitamin A deprivation decreases R1-6 sensitivity by about 2 log units and specifically eliminates the UV peak of this two-peaked receptor (Stark and Zitzmann, J. comp. Physiol 105: 15, 1976). Furthermore in R7, vitamin A deprivation eliminates the afterpotential and inactivation induced by maximal 370 nm R to M conversion. Thus R7 has similar adaptation mechanisms as was shown for R1-6 by Stark and Zitzmann. R7 (as well as R1-6) show vitamin A-dependent separability of photopigment from membrane adaptation processes. The near threshold spectral sensitivity for phototactic preference of Drosophila missing R1-6 has a UV peak not present in mutants missing R7 as well as R1-6; the same spectral sensitivity was obtained in the same experimental conditions from wild-type flies with all receptor systems intact. This shows that R7 inputs directly into phototaxis and can dominate phototactic preference under appropriate adaptation conditions. R7 probably does not input into optomotor behavior since Eckert has found spectral sensitivities for in-flight optomotor behavior in Musca similar with the R1-6 and R8 but not the R7 electrophysiological spectral sensitivities of Harris, Stark and Walker. These and other phototaxis studies indicate that the spectral preference functions obtained by Schümperli (J. comp. Physiol. 86: 77, 1973) which are similar in shape and threshold with electrophysiological R1-6, R7 and R8 spectral sensitivities, show adaptational control of separate receptor input rather than color vision. However, it remains a possibility that R7 may interact with the other retinal color receptors (R1-6 and R8) to mediate color vision as suggested by the results of Quinn, Harris and Benzer (P.N.A.S. 71: 708, 1974), Harris, Stark and Walker, Kirschfeld and Lutz (Z. Naturforsch. 29c: 95, 1974) and Mimura (J. comp. Physiol. 105, 65, 1976).

In summary, R7 is a UV receptor with photoreversible inactivation and direct input into behavior.

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1566 AUTORADIOGRAPHIC IDENTIFICATION OF NEURONS THAT ACCUMULATE TRITIATED GABA IN THE CAT SUPERIOR COLLICULUS. P. Sterling and R. F. Spencer. Dept. Anat., Univ. Penn. Sch. Med., Philadelphia, PA. 19174.

Electrical stimulation and receptive field studies have demonstrated that the retinal and visual cortical inputs can activate inhibitory mechanisms in the superior colliculus of the cat. Nothing is known about which elements in the superior colliculus are inhibitory or what neurotransmitter might be involved. It is known from biochemical studies, however, that the putative inhibitory neurotransmitter GABA is present in high concentrations in the superior colliculus of several mammalian species. In some systems, e.g., the cerebellum, elements that utilize GABA as a transmitter have powerful uptake mechanisms for exogenous GABA. In the present study, this uptake mechanism has been utilized to autoradiographically identify and localize neurons and neuronal processes in the cat superior colliculus.

Tritiated GABA was injected into the superior colliculus of anaesthetized cats pretreated with aminooxyacetic acid, which inhibits the catabolism of GABA by GABA-transaminase. One hour after the injection, the cats were sacrificed by intracardial perfusion of aldehyde fixatives, and sections of the superior colliculus were processed for light and electron microscope autoradiography. Light microscope analysis of 1 μm sections showed that the distribution of label did not form a gradient corresponding to the site of the injection, but rather showed a specific laminar pattern independent of the depth of the injection. The zonal layer was relatively free of label. The superficial gray was heavily labelled throughout, with a dense band in the upper region corresponding roughly to the zone of densest termination of retinal axons. Small cells in this region were frequently densely labelled, intermingled with other small unlabelled cells. Other labelled cells were scattered throughout the depth of the superficial gray, and in the optic layer and intermediate gray as well. Electron microscope analysis of ultrathin sections including the zonal layer and the superficial gray revealed that labelled cells previously identified by light microscopy were small neurones, approximately 10 μm in diameter and characterized by large convoluted nuclei and a rather extensive Golgi apparatus arranged in the sparse surrounding cytoplasm. In some cases, grain clusters were observed to overlie the primary dendrites emanating from the soma of a labelled neurone. In the neuropil of the superficial gray, labelled structures included axon terminals and dendrites containing synaptic vesicles, as well as some dendrites which contained no vesicles in the section. Grain clusters were not seen to overlie retinal terminals. A number of thin myelinated axons were also labelled. There was also evidence for uptake of the tritiated GABA by glia and glial processes.

In conclusion, there are small neurones in the superficial, optic and intermediate gray layers that differentially accumulate exogenous GABA. Such cells are particularly numerous in the upper superficial gray and form one class of cells that gives rise to dendro-dendritic synapses. These results suggest that GABA may be one neurotransmitter involved in mediating intrinsic inhibitory mechanisms in the cat superior colliculus.

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1567 ANTEROGRADE AND RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE FROM THE EYES OF SOME REPTILES. Ruu-Tong Wang*, David R. Colman* and Mimi Halpern. Dept. Anat., Downstate Med. Ctr., SUNY Brooklyn, N.Y. 11203.

Intraocular injection of horseradish peroxidase (HRP, type VI, 0.1 to 8.0 µl of 50% solution in saline) into the vitreous body of garter snakes (Thamnophis sirtalis and Thamnophis radix) results in orthograde and retrograde transport of the enzyme to visual system structures in the CNS. Snakes survived 24 hours to 5 days following eye injections but maximum localization of the enzyme in the CNS was observed in animals surviving 2 to 3 days. Anterograde transport was revealed by the o-dianisidine incubation technique (Colman et al., Brain Res. 102:156, 1976) as blue-green reaction product granules in the contralateral lateral geniculate complex, nucleus lentiformis mesencephali, nucleus posterodorsalis, nucleus geniculatus pretectalis, basal optic nucleus and superficial layers of the tectum. A small amount of HRP was also observed in the ipsilateral lateral geniculate nucleus and nucleus posterodorsalis. Sections processed using 3-3' diaminobenzidine tetra HCl (Graham and Karnovsky, J. Histochem. Cytochem. 14:291, 1966) also revealed the retinofugal system, but less intensely and not sufficiently to describe the terminal fields.

The nucleus of the ventral supraoptic decussation (NVSOD) (Halpern & Frumin, J. Morph. 141:359, 1973) lies medial to the ventral portion of the optic tract in the ventrolateral posterior diencephalon and medial to the fibers of the ventral supraoptic decussation in the lateral, rostral mesencephalon. The cell bodies of this nucleus contralateral to the injected eye accumulate HRP after intravitreal injections but not following extraocular (intraorbital) injections. These cell bodies contain a dense accumulation of green (o-dianisidine incubation) or brown (diaminobenzidine incubation) granules. Injection of HRP in the extraocular (intraorbital) space results in retrograde transport of HRP to the cell bodies of the IIIrd, IVth, and VIth cranial nerve nuclei, but no accumulation of HRP in the nucleus of the ventral supraoptic decussation.

These findings suggest that the NVSOD is the source of efferent fibers to the eye of garter snakes. The absence of HRP in the NVSOD after extraocular injections suggests that this nucleus does not send efferent fibers to some extraocular muscle or gland.

Two additional reptiles (common cape girdled lizard and San Francisco alligator lizard) have been subjected to intraocular injections of HRP. In each case a nucleus has been identified which accumulates HRP in a retrograde manner. The location of this nucleus varies, however, being situated adjacent to the rostral potion of the optic tract in the common cape girdled lizard but located in the caudal mesencephalic tegmentum in the San Francisco alligator lizard.

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1568 STRIATE CORTEX AND VISUAL LOCALIZATION IN THE TREE SHREW (TUPAIA GLIS). Jeannette P. Ward and Marilyn E. Moss*. Dept. of Psychology, Memphis State University, Memphis, TENN. 38152

Lashley's (1943) studies of maze learning with destriate rats established the involvement of visual cortex in spatial function. More recently, auditory cortex has been shown to have a critical role in auditory localization but only under conditions of testing in which a locus approach is the required response. Motor (Heffner & Masterton, 1975) and memory (Ravizza & Diamond, 1974) requirements of the locus approach task have been implicated in the localization deficit consequent to auditory cortex lesions.

The purpose of this study was to examine the possibility of a functional parallel between auditory and visual cortices in the mediation of spatial localization by employing the auditory locus approach paradigm to test the visual localization capacity of destriate tree shrews. This species was the subject of choice because loss of striate cortex results in minimal deficits in basic visual capacity (Snyder & Diamond, 1968; Ward, Frank, & Moss, 1975).

Four intact and four destriate tree shrews were trained for food reward in a semicircular six-choice apparatus. All animals were initially trained to approach a compound visual-auditory stimulus (panel light and white noise) which was terminated following the correct response of entering the one of six doors which was unlocked. When discrimination of the compound stimulus was established and performance stabilized, groups were split and transferred to either an auditory-alone or visual-alone condition. Transfer from the compound to visual-alone stimulus was perfect for all tree shrews both intact and destriate, while all tree shrews in the auditory-alone condition went to chance performance.

With immediate establishment of the criterion performance of 90% correct for two consecutive sessions in the visual-alone condition, evaluation of the memory component of the localization task was begun by the gradual introduction of a delay between the stimulus offset and response completion until stimulus offset and release of the animal were simultaneous. Under these conditions all tree shrews maintained a high level of performance. Further increases of 0.5, 1.0, and 2.0 sec in the delay interval placed the stimulus offset prior to the release of the tree shrew. A constant stimulus duration of 2 sec was maintained for all delays. Intact and destriate tree shrews performed similarly under the delay conditions: both groups showed a marked decline in performance at the 0.5 sec delay and went to chance (20% correct) when the delay between stimulus offset and release reached 1.0 or 2.0 sec. The consistently poor performance of tree shrews in the auditory-alone condition demonstrated that this discrimination was more difficult than the visual discrimination and therefore not a satisfactory control for this species. These four tree shrews were switched to the visual-alone condition where they achieved criterion immediately. Their subsequent performance on all tests was identical to tree shrews tested initially in the visual-alone condition.

Spatial localization of visual stimuli with or without an imposed delay is not impaired in the tree shrew following lesions limited closely to striate cortex. Differences in task requirements or in species may account for the undisturbed spatial function in destriate tree shrews as compared to Lashley's destriate rats.

1569 COMPARISON OF RETINO-TECTAL PATHWAYS IN NORMAL AND SIAMESE CATS: AN AUTORADIOGRAPHIC ANALYSIS. J.T. Weber and J.K. Harting. Department of Anatomy, University of Wisconsin, Madison, WI. 53706.

The autoradiographic tracing method was used to study the organization of retino-collicular pathways in normal and siamese cats.

In the normal cat the entire rostral-caudal extent of the contralateral colliculus contains transported protein. This contralateral protein is limited to the superficial tectal layers, and is most dense within a band which occupies the dorsal portion of the stratum griseum superficiale (SGSI of Kanaseki and Sprague '74). The density and pattern of contralateral label differ within portions of the colliculus containing the representation of central vision. In particular, the dense band of label within SGSI is thinner and at some points discontinuous. The label within more ventral regions of the stratum griseum superficiale is markedly reduced, however, a few dense patches are present within these deeper regions. Transported protein within the ipsilateral colliculus is considerably reduced and does not reach the rostral and caudal poles. Most significantly, the label occurs in patches or puffs (Graybiel '75) which are most prevelent within the middle of the stratum griseum superficiale (SGS2 of Kanaseki and Sprague '74).

The pattern of transported protein within the colliculi of siamese cats differs in two respects from that seen in normal cats. First, within that region of the contralateral colliculus immediately caudal to the representation of the vertical meridian, a series of horizontal bands or leaflets of label are apparent. These bands lie immediately ventral to the dense band within SGSI. We feel that these leaflets represent the axon terminals of ganglion cells located within the contralateral temporal retina.

Second, the amount of ipsilateral label is somewhat reduced when compared to that seen in normal cats. This reduction is most apparent within the region of the colliculus immediately caudal to the representation of the vertical meridian, i.e. the same region which contains the series of leaflets or bands contralaterally. Caudal to this region, the amount of ipsilateral label increases dramatically and occurs in patches or puffs similar to that seen in normal cats.

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1570 RECEPTOR-HORIZONTAL CELL CONNECTIONS IN THE GRAY SQUIRREL RETINA

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The squirrel retinas as a group recently have been shown to possess rod as well as cone photoreceptors. Because these rods have many cone-like features it is of interest to determine whether the receptor-horizontal cell connections are the same as those in mammalian retinas which have rods with a more conventional appearance. The gray squirrel retina was chosen for this determination because, among the squirrel retinas, it has a relatively high proportion of rods. The Golgi method reveals two morphologically distinct types of horizontal cell in the gray squirrel retina - type H1 which has an axon and type H2 which does not.

H1 has a prominent, round 8-10 um soma confined to the outermost row of somata in the inner nuclear layer. The dendrites leave the soma at multiple points and ascend to the receptors in a 35-50 um tuft. Golgi-EM shows that the dendrites invaginate only cones. The dendrites constrict upon entering the cone bases and then enlarge to 0.5-1.5 um to become the lateral elements of triads. The axon originates from either the soma or one of the main dendrites and runs through the outer plexiform layer in a relatively constant direction for several hundred microns. The axon occasionally branches, sometimes as early as 100 um from the soma, but elaborate terminal structures have not been observed. Along its entire length the axon sends slender sprouts (about 10/100 um) to the receptors where they terminate in 0.5-1.0 um swellings within the receptor bases. Golgi-EM shows that these sprouts invaginate only cones and the swellings can often be identified as the lateral elements of triads.

H2 has a radially elongated 7-9 um soma which is usually located at the outer margin of the inner nuclear layer but with more variance in depth than the H1 somata. The soma gives rise to only a few major stems at its apex from which the dendrites branch over a 120-190 um field. The dendrites are thick and varicose, ranging from 0.2-1.5 um diameter. Along their length are larger 2-3 um swellings which are either continuous with the dendrites or are isolated by slender stalks. Golgi-EM shows that these swellings abut or invaginate both rod and cone bases. Participation in triads was not observed in the one Golgi-impregnated cell that was sampled but non-Golgi-impregnated processes of similar large size have been seen as lateral elements of triads in both rods and cones. H2 also sends slender stalks to receptor bases where they invaginate and swell to 0.5 um. These Golgi-impregnate much less frequently than the larger swellings and were not characterized by EM. Within the limits of light microscopy, the small swellings apparently also contact both rods and cones.

This picture departs significantly from that of retinas which have more conventional rods. Both the cat and monkey retinas have a horizontal cell type which is similar to H1. However, while their dendrites contact only cones, the axons have elaborate terminals which contact only rods. The cat retina has an axon-less cell type which is similar to H2. However, this type has been reported to contact only cones.

1571 THE ANALYSIS OF MOTION AND COLOR IN THE POSTERIOR BANK OF THE SUPERIOR TEMPORAL SULCUS. S.M. Zeki* (SPON: R.W. Guillery). Anatomy Department, University College London, London, England.

In the rhesus monkey, the posterior bank of the superior temporal sulcus forms part of the prestriate visual cortex. In its upper part, there are two regions of this sulcus, one situated medially and the other laterally, which have separate callosal connections (Zeki, 1970). We studied the afferent inputs to these two regions anatomically in experiments where the corpus callosum was sectioned and labelled amino acids were injected into other visual areas. By this method, it was found that area 17 projects to that part of the superior temporal sulcus occupied by the more medial of the two callosal inputs. By contrast, the part of the sulcus occupied by the more lateral callosal input was found to receive a strong projection from the anterior bank of the lunate sulcus, an area rich in color coded cells. We conclude that at this level there are at least two distinct regions within the posterior bank of the superior temporal sulcus, each with its own independent afferent and callosal connections.

We have recorded from single cells in the superior temporal sulcus in animals in which the corpus callosum had been sectioned previously. The degeneration produced by the corpus callosum was used to provide anatomical landmarks enabling us to assign cells to the lateral or medial areas of the sulcus. Such recordings revealed that receptive fields in the lateral part of the sulcus were topographically organized and that most of the cells were color specific. Some of these color coded cells had opponent properties whereas others did not, even though they were unresponsive to white light. Action spectra revealed a wide distribution of sensitivities with some cells being sharply tuned and responding only over a 40nm segment of the spectrum and others having relatively broad curves.

By contrast cells recorded from in the region of the more medial callosal patch within this sulcus were directionally selective, without any obvious color coding.

These combined anatomico-physiological experiments show that there are at least two different regions in the superior temporal sulcus which have different afferent connections and which contain different populations of functional cell types.

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*Royal Society Henry Head Research Fellow.

Zeki, S.M. (1970) Brain Res., 19, 63-75.

1572 SPECIFICITY OF SEROTONINERGIC RECEPTORS MEDIATING INHIBITION IN LIMULUS LATERAL EYE. Alan R. Adolph. Eye Research Institute of Retina Foundation, Boston, Mass. 02114.

Formaldehyde fluorescence histochemical studies(Adolph and Ehinger, Cell Tiss. Res. 163:1, 1975)have shown that retinal eccentric cell synaptic processes in the neuropil of Limulus lateral eye, have a strong uptake mechanism for extracellular serotonin(5-hydroxytryptamine;5-HT), and its isomer and structural analogue indoleamines, 6-hydroxytryptamine(6-HT) and 5, 6-dihydroxytryptamine(5, 6-DHT). This uptake mechanism may function to inactivate the putative 5-HT transmitter mediating lateral inhibition in the eye(Adolph, J. Gen. Physiol. 67:417, 1976). Focal application of 6-HT and 5, 6-DHT in alternation with 5-HT from double-barrel iontophoretic electrodes, or alternating gross perfusion of the retinal preparations by these substances showed, however, that membrane receptors activating the inhibitory response were structurally specific for 5-HT and unaffected by the isomeric analogues. Focally applied 5-HT, in a neuropilar environment in which the concentration of isomer/analogue was maintained consistently high by perfusion, continued to interact with receptors and evoke inhibitory responses. The lack of direct and cross-effects of even the "structurally overlapping" analogue, 5, 6-DHT emphasizes the high specificity of the inhibitory transmitter receptor for 5-HT. Within the neuropil, axonal membrane of the same eccentric cell contains both pre- and post-synaptic areas, often with autosynapsis(self-inhibitory contacts) and intercellular, lateral inhibitory, synaptic contacts. This may indicate extreme heterogeneity and localization of transmitter-receptor and uptake sites.

1573 RECEPTIVE FIELDS IN AREAS 18 AND 19 OF THE AWAKE, BEHAVING MONKEY. Joan S. Baizer. Lab of Neurobiology, National Institute of Mental Health, Bethesda, Md. 20014.

In the posterior bank of the lunate sulcus folded back under area 17 (area 18) we have previously described six different receptive field classes. Receptive fields of these cells are only slightly larger than those of area 17 cells at comparable eccentricities. In the cortex lying more anterior and deeper in the lunate sulcus (area 19), there is a dramatic increase in receptive field sizes. Most commonly encountered are large field orientation cells, large field directionally selective cells, and a new class of cells showing neither orientation nor direction specificity and giving sharp on and off responses to large, flashed stimuli. These results suggest that there is a second, functionally distinct visual area in the lunate sulcus. Some cells in this area perform qualitatively similar analyses of visual information as cells in areas 17 and 18, but with a different degree of resolution. (Supported by an NIMH Postdoctoral Fellowship.)

1574 INTENSITY CODING IN THE VISUAL SYSTEM OF THE AWAKE MACAQUE. R. B. Barlow, Jr., D. M. Snodderly, Jr., and H. A. Swadlow*. Eye Research Institute of Retina Foundation, Boston, MA, and Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Changes in the diameter of the pupil and in the discharge of LGN cells of the awake macaque were measured over a wide range of light intensity. Ganzfeld flashes of white light were delivered under dark-adapted conditions to a surgically immobilized eye while the other eye was observed in the infrared. Three-sec flashes elicited a consensual reflex that was first detectable for intensities about 1.4 log units above human visual threshold and was graded over at least an 8 log-unit range from -5 to 3 log mL. Shorter flashes (0.1 to 0.5 sec) but otherwise identical conditions elicited a graded response in some LGN cells in the same monkey. These cells elicited detectable responses at -2 log mL (human photopic threshold) and discharged increasing numbers of spikes from -2 to 3 log mL which corresponds roughly to the range of human photopic vision. Over this range response latencies decreased monotonically from 230 to 35 msec. These cells showed no evidence of rod input. Similar functions relate responses of these cells and pupil reflex to light intensity. Other LGN cells had narrower ranges and some had nonmonotonic response functions which extended over a 7 log-unit range. The pupil data indicate that intensity coding in the optic nerve fibers of the awake macaque is graded over a range of at least 8 log units. However, only in the upper 5 log units of this range is intensity coding readily apparent in the discharge of single LGN cells. The 5 log-unit range is the widest yet reported for the response of LGN cells to diffuse illumination. We note that such wide-range coding occurred in the macaque visual system under stimulus conditions which yielded evidence for wide-range intensity coding in human psychophysical experiments.

1575 AUTORADIOGRAPHIC LOCALIZATION OF CHOLINERGIC CELLS IN RETINA. R.W. Baughman*, C.R. Bader*, and E.A. Schwartz* (SPON: Z.W. Hall). Dept. of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115.

The presence of acetylcholine, choline acetylase and acetylcholinesterase in the vertebrate retina and the ability of the retina to take up radioactively labelled choline and convert it to acetylcholine are well established. In this study the release of ^{3}H acetylcholine and the localization of cells that accumulate ^{3}H choline was investigated. In red-eared turtle and white Leghorn chick retina newly synthesized ^{3}H acetylcholine was released in vitro by increasing the extracellular K^{+} concentration (10mM) or, in the turtle, by a flashing light. Both types of release were blocked in a Ca^{++}-free medium containing 20mM Mg^{++} or 2mM Co^{++}. Recently taken up ^{3}H choline and synthesized ^{3}H acetylcholine, present in about a 1:1 ratio were localized in chick and turtle retina by means of autoradiography. After a 20 min. in vitro incubation with $5x10^{-6}$ M ^{3}H choline a piece of retina was freeze dried, fixed in the vapor phase, embedded in epoxy resin, sectioned and coated with a dry film of nuclear track emulsion. In the resulting autoradiographs, grains were localized over cells in the inner nuclear and ganglion cell layers, and in two well defined bands in the inner synaptic layer. Very little label was present in the outer synaptic layer, photoreceptor layer or optic nerve fibers. In a sample section, 5% and 14% respectively of the cells in the inner nuclear and ganglion cell layers were labelled. In the inner nuclear layer, half of the labelled cells were aligned in the middle part of that layer while the other half was located immediately adjacent to the inner synaptic layer. Thus a small number of retinal cells, possibly including bipolar, amacrine, and ganglion cells, accumulate ^{3}H choline. Unless there is participation of efferent fibers, one or more of these cell types synthesizes acetylcholine from choline and releases acetylcholine under conditions consistent with synaptic release.

1576 RETINAL GANGLION CELL RESPONSE DISTRIBUTIONS TO FLASHED LIGHT SPOT STIMULI. Richard Binggeli. Dept. of Anatomy, University of Southern California School of Medicine, Los Angeles, California 90033.

Repetitive responses to flashing stationary spots of varying size, contrast, and color were recorded from pigeon ganglion cell axons. The responses were rigorously quantified to yield mean firing for individual cells to the ON and the OFF of the stimulus. Cell values were grouped for each stimulus condition, the group means calculated, and the distributions plotted. Light and dark spots of three sizes ($\frac{1}{2}^{o}$, 2^{o}, and 4^{o}) in five colors as well as a full field flash were used. As previously reported for small spots, the average spike output is considerably smaller than the typical mammalian response. Grouping of responses showed random, continuous distributions to all color-spot stimuli. Many difference functions (e.g. ON response minus OFF response) and correlation scattergrams also showed continuous distributions with little indication of subgrouping or clumping. These results argue against the grouping of ganglion cells according to functional classes using the stimulus variables reported here. The mean responses to large ($>20^{o}$) and small (0.5) spots were previously reported (1.4 to 2.9 impulses at the ON respectively and .7 to 2.0 at the OFF). The response to intermediate (2^{o} and 4^{o}) spots were measured. The 2^{o} spots, both light and dark, produced a smaller ON than the large and 0.5^{o} spots while the OFF responses were intermediate in size. Considering the total group of ganglion cells as a single population, modeling was developed to account for the results. The present data are compatible with a model which contains only vertical inhibition and laterally projecting excitatory influences.

1577 A QUANTITATIVE STUDY OF PYRAMIDAL CELL SPINE DENSITY IN DEVELOPING VISUAL CORTEX OF NORMAL AND DARK REARED MACAQUE MONKEYS. R.G. Boothe and J.S. Lund. Dept. of Ophthalmology, Univ. Wash., Seattle 98195.

Qualitative observations from golgi stained material have revealed that neurons in monkey (Macaca Nemestrina) visual cortex (area 17) undergo a marked proliferation of dendritic spines during their early postnatal development. This spine rich stage is followed by a marked reduction in the number of dendritic spines to the adult level. In order to study the time course of this spine development quantitatively counts of spines have been made from apical dendrites of Layer III pyramidal cells at several postnatal ages. Counts were made on ten micron segments sampled every 50 microns from the proximal to distal ends of the apical dendrites. Spine density along the apical dendrite increases by as much as 400-500 percent between the ages of birth and two months postnatal. Then between the ages of two months and adult, spine density decreases by as much as 50 percent along the proximal portion of the dendrite and as much as 75 percent distally. This spine loss occurs fairly rapidly between two months and six months of age. However, even at nine months of age spines are more dense than in the adult. In a monkey reared in continuous darkness from two weeks to three months of age, spine density is significantly lower than in a normally reared three-month old animal, and resembles the spine density of a normally reared monkey three to five weeks old. It is suggested that in the normal monkey during the "critical period" there is a rapid acquisition of synapses by these pyramidal neurons in the first two postnatal months followed by a period of considerable loss of contacts. The critical period may involve not only an acquisition of contacts, but also their selective elimination.
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1578 EFFECTS OF PICROTOXIN AND STRYCHNINE ON RECEPTIVE FIELDS OF RABBIT GANGLION CELLS. John H. Caldwell* and Nigel W. Daw. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis,MO,63110.

It has been shown that direction selective and orientation selective ganglion cells lose their selectivity if picrotoxin is added to the retinal blood supply but remain selective if strychnine is added. On the other hand, the local edge detector ganglion cells lose their size specificity in the presence of strychnine but not picrotoxin (Wyatt and Daw, Science 191:204,1976; Caldwell and Daw, ARVO 1976).

There are eight types of center-surround ganglion cells in the rabbit (similar to the X, Y, and W or brisk-sluggish/transient-sustained cells described in the cat). We have been studying the effects of picrotoxin and strychnine on each of these. The effects are less striking than those seen with the more complex ganglion cells described above and in no case has either the center or the antagonistic surround been abolished.

The effects of drugs upon more detailed aspects of the receptive fields are also being studied.

1579 RESPONSE PROPERTIES OF RETINAL GANGLION CELLS IN THE SIAMESE CAT. Yuzo Chino, M. Shansky and D. I. Hamasaki*. Illinois College of Optometry and Bascom Palmer Eye Institute.

Single unit recordings were made from the optic tract of four Siamese cats, and the response properties of 136 retinal ganglion cells were studied. In particular, we studied the misrouted retinogeniculate fibers which originate primarily from a vertical strip of retina approximately 20-25° in width just temporal to the area centralis.

The distribution of X- and Y-cells was determined in 66 units from two Siamese cats with a contrast reversal stimulus. 73% of the cells were X- and 27% were Y-cells. Of the 20 misrouted fibers, 85% were X- and 15% were Y-cells. The average size of the receptive field center did not differ significantly between the normally and aberrantly projected retinal fibers. In addition, no consistent relationship was observed between the interocular alignment of the eyes and the response properties in spite of the varying degree of convergent and divergent interocular alignment exhibited by these animals.

We conclude that the normal and misrouted retinogeniculate fibers in the Siamese cat have similar properties.

1580 CONES SURVIVE RODS IN THE LIGHT-DAMAGED EYE OF THE ALBINO RAT. Carol M. Cicerone*. (SPON: J. Bradley Powers). Vision Research Laboratory, Dept. of Ophthalmology, University of Michigan, Ann Arbor, MI 48109.

Previous work shows that in rat continuous exposure to intense light causes photoreceptor destruction which is accompanied by a drastic decrease in the amplitude of the electroretinogram (ERG). Recent behavioral studies on these animals whose retinas were apparently without intact rods show that they can nonetheless perform pattern discrimination tasks. What remaining retinal structure now serves the (rod) photoreceptor function? It has long been acknowledged that the rat's eye possesses cones, but the cones are so few that often little physiological function is ascribed to them. However, persistent and recently clear-cut physiological evidence shows that the vision of rats is not solely determined by the rhodopsin rods. Here, I present two lines of evidence that continuous exposure to intense light first damages the rhodopsin-filled rods while sparing the photopic mechanisms in the albino rats. First, the return in the dark of the ERG sensitivity after 60 seconds of bright light adaptation was measured. In the normal albino, the dark adaptation curve is composed of two branches. The spectral sensitivity of the mechanism underlying the late branch is consistent with rhodopsin rods. The spectral sensitivity of the early branch shows a shift toward longer wavelengths. After light damage, the same animal's dark adaptation curve shows a large threshold increase for the rod-mediated portion with a small threshold increase for the cone-mediated portion. Second, with the scotopic system damaged by light, chromatic adaptation reveals the different photopic mechanisms. Three different photopic mechanisms with spectral sensitivities conforming to single pigment nomograms with peaks at 450, 510, and 560 nm are required to fit the data. Thus, it is likely that cones mediate vision in light-damaged rats. (Supported by PHS postdoctoral fellowship EY 02161 and grant EY 00379 to D. G. Green.)

1581 OPTIC NERVE DEFORMITIES IN A STRAIN OF ALBINO RAT. Richard K. Cooley* and R.W. West (SPON: C. Harley). Dept. of Psychology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

Adult Sprague Dawley albino rats from a major Canadian supplier (Canadian Breeding Farms, Lapaire, Quebec) have recently shown a high incidence of abnormal trajectories of their optic nerves. Within separate shipments the defect has ranged in order of increasing severity from normal appearance to one or both of the optic nerves being kinked, folded back on themselves, or widely looped. There did not seem to be a marked tendency for the nerve on one side to be more frequently or severely deformed than the other and the defect was seen in both sexes. A summary of the defects in two typical shipments of male rats spaced in time is shown below.

	JULY '75	MARCH '76
NORMAL	1	7
ABNORMAL	33	16
right	8	3
left	9	13
bilateral	16	0

The July '75 group showed extreme abnormalities which tended to be bilateral. The March '76 group showed less severe defects which took the form of a kinked or "inch worm" appearance which was unilateral. Informal observations during previous work with the hooded, Long Evans rat from the same supplier showed similar defects in not more than 5 out of 400-500 animals. These Sprague Dawley rats may be of use for studying the development of the visual system. Also, because these rats have been widely used in Canadian research it is of particular importance to determine whether this defect includes abnormal visual projections.

1582 THE EARLY STAGES OF DARK ADAPTATION IN LIMULUS EYE. George E. Corrick* and Conrad G. Mueller* (SPON: H.D. Potter). Dept. of Psychology and Center for Neural Sciences, Indiana University, Bloomington, IN 47401.

The sensitivity and responsivity during the first ten sec of dark adaptation were studied in excised lateral eyes of Limulus by recording single unit activity in the optic nerve. After adapting to a steady level the adapting light was turned off periodically and a brief test flash delivered at intervals between .1 sec and 10 sec after the cessation of the adapting light. Using this paradigm two experiments were conducted: (1) Determination of the threshold intensity required for the test flash to elicit only one impulse; (2) Measurement of the number of impulses in response to a constant intensity test flash. The results indicate that receptor sensitivity and responsivity do not simply increase monotonically as a function of time in the dark. A plot of sensitivity over the interval investigated is approximately U-shaped. After the end of the adapting stimulus sensitivity initially decreases, reaching a minimum about 1-2 sec into dark adaptation. Sensitivity then begins an increase that continues until dark adaptation is complete. The manner in which the number of impulses elicited by a constant intensity test flash changes as a function of time in the dark is also U-shaped; it shows a time course qualitatively similar to, but quantitatively different from, the sensitivity curve. These results will be discussed in the light of changes in the function relating number of impulses and stimulus intensity during early stages of dark adaptation.

1583 ASYMMETRIC PATTERN-EVOKED POTENTIALS IN HUMAN ALBINOS. Donnell Creel, Richard A. King*, Carl J. Witkop, Jr.* and A. N. Okoro* Neuropsychol. Res., V.A. Hospital, Salt Lake City, Ut. 84113; Human and Oral Genetics, Univ. Minnesota Dental and Med. Sch., Minneapolis, Minn. 55455; and Dept. Dermatology, Univ. Nigeria Teaching Hospital, Enugu, Nigeria.

Recent electrophysiologic and anatomic studies indicate that the nondecussated optic system is disorganized in albino human beings similarly to the anomalous organization previously documented in albino members of eight other species of mammals. Members of 70 families which included 130 albinos were examined in Enugu, Nigeria. Seventy of these albinos were of the tyrosinase positive type with yellow hair, freckles, pigmented nevi, and blue eyes exhibiting variable monocular esotropia and nystagmus with a major rotatory component. There was some iris pigment, usually golden in color mostly at the pupillary border forming a starburst appearance over the iris, but no retinal pigmentation. Visually evoked potentials were recorded from 27 of these albinos and 10 normally pigmented Nigerians. Diffuse flash and a 15' checkerboard pattern were used as stimuli. There was a significant asymmetry between the evoked potentials recorded from each hemisphere of monocularly illuminated albinos, as compared to the normally pigmented group of controls. The extent of the hemispheric asymmetry varied considerably between albinos. The variability in the retinogeniculostriate projections appears to be greater in human albinos than in other albino mammals. We suggest that the variance in degree of nystagmus observed between human albinos of similar pigmentation and clinical type is a function of the variance in the optic projections to the pretectum and midbrain.

1584 THE EFFECTS OF VISUAL DEPRIVATION ON THE CAT SUPERIOR COLLICULUS. Max Cynader, Dept. Psychol., Dalhousie Univ., Halifax, Nova Scotia

The response characteristics of single cells in the superior colliculus have been examined in cats which have been reared under conditions of atypical visual exposure. In normally-reared cats, single units in both the superficial and deeper layers of the colliculus are visually excitable and frequently direction selective. In addition, units in the deeper layers can be activated by auditory and/or tactile input. Following total visual deprivation, single units in the superficial layers of the colliculus can still be reliably activated by visual stimuli, but selectivity for direction of stimulus movement is abolished. The visual responses of single units in the deeper collicular layers are markedly attenuated or absent, although responses from other sense modalities can still be obtained. These results suggest that the deep layers may be more sensitive than the superficial layers to the effects of visual deprivation and further suggest a competitive process among the different sense modalities for control of the responses of the deep collicular neurons. This may be analagous to the competition for cortical neurons which occurs between input from the two eyes following monocular deprivation.

The increased experiential susceptability of the visual input to the deeper layers of the colliculus is also observed following monocular deprivation. If kittens are monocularly deprived for short periods of time and recordings are made in the colliculus contralateral to the deprived eye, vigorous responses through both eyes are observed in the superficial layers, but responses from the deprived eye are absent or markedly attenuated in the deep layers.

1585 VISUAL EVOKED POTENTIALS IN PRESTRIATE AND INFEROTEMPORAL CORTEX SENSITIVE TO SHAPE OF THE EVOKING STIMULI. Benj. Dawson and Leo Ganz, Dept. of Psychology Stanford University, Stanford, CA. 94305.

Neurons in inferotemporal (IT) cortex respond to specific visual stimuli (e.g., Gross, et al., J. Neurophysiol. 35: 96, 1972), but responses specific to different visual stimuli have not been found using gross electrodes (Vaughan and Gross, Exp. Brain Res. 18: 19, 1969; Gerstein, et al., JCPP 65: 526, 1968). Four rhesus monkeys performed match from sample tasks. In the first task, a single circle (○) or four smaller circles (⁘) were briefly (.1 msec) presented as a sample stimulus, and .5 sec later both the single circle and the four smaller circles stimuli were presented on side panels as possible matches. In the second task, horizontal and vertical stripes (☰,|||) were used instead of circles. These tasks have both visual and mnemonic aspects. Fine wire (100 μ diameter), bipolar electrodes recorded the potentials evoked in striate, prestriate and IT cortices by the brief sample stimuli. Significant differences in the potentials evoked by different stimuli were found in all areas recorded from. Each electrode showed a different pattern of sensitivity -- for example, an electrode might record large differences between circle stimuli, but not between stripe stimuli. The level of significance of these form specific differences increased as one progressed anteriorly from striate into IT cortex, with a slight decrease at the most anterior IT cortex electrodes. This confirms microelectrode work in suggesting a visual or mnemonic function for these areas, and suggests an increasing analytic sophistication of more anterior cortices.

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1586 SHORT WAVE-LENGTH RESPONSES MEDIATED BY INPUT FROM RED-SENSITIVE CONES IN FOVEAL GANGLION CELLS OF MACAQUES. F.M. de Monasterio* and P. Gouras. National Eye Institute, NIH, Bethesda, Md 20014.

About 10% of the cells in a reference sample of 487 colour-opponent ganglion cells of the central 20° of the rhesus monkey retina had short and long wave-length responses mediated by red-cone input, whereas mid-spectral responses received green-cone input. Responses at the far blue (400-420 nm) were enhanced by a blue background and depressed by yellow, red and magenta backgrounds; these responses persisted on white backgrounds markedly depressing rod sensitivity. In addition to opponent green-cone input, these responses were antagonised by concealed blue-cone input, mostly in parafoveal cells. These cells predominated in the centre of the fovea (0-0.5°: 20%) and diminished with increasing retinal eccentricity (10-20°: 2%); they projected to the lateral geniculate nucleus, but not to the superior colliculus. Their retinal distribution and the relative sensitivity of their far-blue responses parallelled the macular pigment distribution, but had a reciprocal relationship with those of cells receiving blue-cone input. The sensitivity ratio between the short and long wave-length threshold minima is similar to the one expected between the β and α bands of an A_1 pigment, taking lens absorption into account.

These cells were recorded in eyes with intact lenses, using different anaesthetics. Far-blue responses were not due to glass fluorescence or filter secondary transmission bands; their retinal distribution was not due to Stiles-Crawford effects.

The chromatic properties and the retinal distribution of these cells were very similar to those of red-hue responses elicited by blue lights in human colour-naming experiments. These cells might provide a basis for the phenomenon of redness to short wave-lengths.

1587 ABSENCE OF RETINOTOPIC ORGANIZATION IN INFERIOR TEMPORAL CORTEX. Robert Desimone* and Charles G. Gross. Dept. Psychol., Princeton Univ., Princeton, N. J. 08540.

Single neurons in the posterior dorso-lateral portion of inferior temporal cortex of the macaque monkey have visual receptive fields that almost always include the fovea, are large (median area = 409 degrees^2), and usually (60%) extend across the vertical meridian well into both visual half-fields (J. Neurophysiol. 35:96, 1972). To determine whether inferior temporal cortex is retinotopically organized we plotted receptive fields over its entire extent with multi-unit electrodes in eight monkeys. We found that throughout inferior temporal cortex, including its ventral and anterior aspects, the receptive fields almost always included the fovea and appeared similar in size and laterality to those recorded previously from the posterior dorso-lateral region. There was no evidence of a retinotopic organization within inferior temporal cortex. Although neurons in inferior temporal cortex were always modality specific, we did find a polysensory area just dorsal to inferior temporal cortex in the superior bank of the superior temporal sulcus which was responsive to visual, auditory, and somesthetic stimuli.

1588 AMMONIUM ACETATE REVERSAL OF EXPERIMENTAL AMBLYOPIA, F.H. Duffy*, J.L. Burchfiel*, and S.R. Snodgrass*(SPON: A.V. Lorenzo) Dept. of Neuroscience, Children's Hospital and Harvard Med. Schl., Boston, MA. 02115

We have reported that intravenous (IV) bicuculline, a GABA receptor blocker, can reverse the lack of binocular input to visual cortical neurons in cats with experimental deprivation amblyopia (Nature 260:256,1976). IV bicuculline is a toxic drug, its duration of action is very brief, and not all neurons respond to it in our experience. Picrotoxin appears to have similar liabilities. Lux (Exp Brain Res 11:431,1970) and others have reported that ammonium salts, given IV or by iontophoresis, can antagonize chloride dependent synaptic inhibition by a non-receptor mechanism. We gave IV infusions of ammonium acetate (1-4 mmol/kg, adjusted to pH 7.4) to cats with experimental deprivation amblyopia, produced by monocular lid suture in the first weeks of life. After initial anesthesia with a short-acting barbiturate and refraction, extracellular unit activity was studied in area 17 of paralyzed, locally anesthetized cats, with careful monitoring of EEG, pulse and blood pressure. We determined that the characteristic physiological abnormality (Wiesel and Hubel, J. Neurophysiol 26:1003, 1963) was present and then evaluated the responses of cells to visual stimuli after IV bicuculline and then ammonium acetate. Each agent could restore binocularity. In comparison to bicuculline, ammonium acetate produced a longer response (up to 2 hrs), a greater effect upon receptive field properties for the normal eye, and responses in a greater number of cells, including some cells unresponsive to bicuculline. Convulsive discharges were not a problem with ammonium acetate. Ammonium salts appear to be safer and more useful for behavioral studies than is IV bicuculline, their effect supports the postulate that synaptic inhibition is a mechanism of the amblyopic deficit, but they provide no new information about the locus or nature of the inhibitory process.

1589 LOCAL CONTROL OF PHOTOMECHANICAL MOVEMENTS IN FISH RETINA. S. S. Easter, Jr., and Alan Macy*; Div. Biol. Sci. and Neuroscience Program, U. Michigan, Ann Arbor, MI, 48109, and Bermuda Biological Station.

In the retinas of most fish and amphibians, light causes contraction of cone myoids and vitread migration of pigment granules in the pigmented epithelium. The reverse movements occur in darkness.

Experiments were carried out on Cichlasoma biocellatum (the "Jack Dempsey") to determine whether these photomechanical movements are under local or hormonal control. Fish were curarized and their gills irrigated during 2 hours of dark adaptation and subsequent 1.25 hour exposure to a spot of monochromatic light (538 nm) presented in Maxwellian view. The spot subtended 16^{0} visual angle, and covered a retinal region approximately 800 μm in diameter. Paraffin histology with hematoxylin and eosin staining revealed local light adaptation of cones and pigmented epithelium. At all intensities within a four log unit range, the locally adapted area was circular, with a diameter, corrected for histological shrinkage, equal to that of the spot. The entire retina was light adapted above this range, dark adapted below. The boundaries of the region were sharp, with the transition from contracted to extended cones and pigment occupying about 100 μm (approximately 12 cone diameters). These results show that photomechanical movements are neither under systemic hormonal control, nor influenced by light (or a diffusable substance resulting from light) delivered to a site farther than 100 μm from the responding cell. They also suggest that the optical quality of the image is high. Since the diameter of the locally adapted region is constant over a four log unit range of intensity, and the boundary of the region is sharp, the intensity of the stray light must fall off by approximately 10^{3}-10^{4} over a distance of only 100 μm from the boundary of the spot. (Supported by PHS grant EY-00168 to SSE and a special grant from NSF to the Bermuda Biological Station.)

1590 RESPONSE PROPERTIES OF IDENTIFIED VISUAL INTERNEURONES IN THE THIRD OPTIC GANGLION OF DIPTERANS (Phaenicia sericata) IN THE CONTEXT OF BEHAVIOURAL RESPONSES. Hendrik Eckert and Lewis G. Bishop. Dept. Biol. Sciences, USC, Los Angeles, CA. 90007

Intracellular recordings from directionally selective motion detecting fibers (DSMD) to moving striped patterns were obtained, and the fibers were subsequently syained by iontophoretic injection of Procion Yellow. Two types of fibers were encountered, one maximally sensitive to vertical, the other to horizontal pattern movement. Anatomically, the DSMDs are identical with the sets of giant fibers reported previously by Pierantoni (Biocyb. Congress, Leipzig 1973). Comparison of the responses of the horizontal DSMDs (comprising the Horizontal System) to the torque responses of fixed flying flies under comparable stimulation by moving striped patterns shows a remarkable similarity in its dependance on the velocity, contrast frequency and spatial wavelength of the pattern. This is taken as a strong support for the notion that these fibers are intimately involved in the behavioural (optomotor) response and possibly control it.

1591 SUBJECTIVE PHOSPHENE DESCRIPTIONS BY BLIND VOLUNTEERS WITH CHRONICALLY IMPLANTED ELECTRODES. Jerald R. Evans, Wm. H. Dobelle, Michael G. Mladejovsky*, John P. Girvin and T. S. Roberts. Neuroprostheses Program Inst. Biomed. Eng., Univ. of Utah, Salt Lake City, UT 84112 and Dept. of Clin. Neurol. Sci., Univ. and Victoria Hospitals, Univ. of West. Ontario, London, Ontario, Canada N6G 2K3.

Phosphenes are photic sensations produced by electrical stimulation of visual cortex. Blind human subjects report such subjective aspects to these sensations as color, size, brightness, and distance. Coloration of about 3/4 of the phosphenes ranges over subdued hues of red, blue, and yellow, and the remainder are white. The sizes range from pinpoint to a half-dollar at arms' length. Depending on the intensity of stimulation, phosphenes can be barely visible to uncomfortably bright. Distances perceived range from right in the eye to several feet. Also, stimulation of a single electrode can produce multiple or compact phosphenes. These qualities can be correlated with site of stimulation, location in visual field, and stimulation parameters. Studies of these correlations are greatly facilitated by use of an interactive computer system with CRT display. Examples of the findings are presented.

1592 RELAY CELL CLASSES IN THE CAT'S LGNd. David Ferster* and Simon LeVay. Dept. of Neurobiology, Harvard Medical School, Boston, Mass. 02115

Neuronal somata were examined in serial 1μ sections at various eccentricities in the normal cat's LGN, also after localized peroxidase injections in areas 17 and 18, and in cats reared with monocular lid suture. Histograms of cell size were prepared with attention to the presence or absence of cytoplasmic laminar bodies (CLBs).

In laminae A and A1, 3 classes of cells were distinguished. The smallest (class III, diameter 10-25μ, 20% of all neurons) lacked CLBs and were not labelled after cortical injections. They were interpreted as being interneurons. Class II cells (15-30μ) were defined by presence of CLBs. They were all labelled after injection of 17 but not 18. Their numbers declined from the representation of the area centralis (60% of total neurons) to the far periphery (30%), suggesting that they were X-cells (Hoffmann et al., J. Neurophys. 35, 518). Class I cells (20-45μ) were relay cells which lacked CLBs. Their numbers increased with eccentricity, suggesting identity with Y-cells. Most of these projected to 17 only (class Ib), but the very largest cells (class Ia, 35-45μ, 3-5% of total) were labelled after 18 injection only. More cells projected to 18 from A1 than from A. In contrast to previous studies, our results indicate that few if any cells in the A laminae project to both 17 and 18.

An identity between the cells of classes I, II and III and the type 1, 2 and 3 neurons recognizable in Golgi preparations (Guillery, J.C.N., 128, 21) was suggested by a correspondence in sizes. It was confirmed by the observation, in serial semithin sections of Golgi material, that CLBs were present in Guillery's type 2 cells and in no others.

The effect of monocular deprivation on cell size was much more severe for class I than for class II cells. This differential effect parallels the physiological observation of selective loss of Y-cells (Sherman et al., J. Neurophys., 35, 532).

1593 EFFECTS OF MONOCULAR DEPRIVATION ON RECEPTIVE FIELD DEVELOPMENT IN RABBIT SUPERIOR COLLICULUS. Patricia C. Fox, Kao Liang Chow and Amy Schick Kelly* Dept. Neurology, Stanford Univ. Sch. Med., Stanford, CA 94305.

We have investigated the effects of monocular deprivation on the sequence of development of receptive fields of neurons in superior colliculus (SC). One eye of Dutch belted rabbit pups was sutured shut from before normal eye opening until the day of recording. Receptive field properties of normal (contralateral to normally opened eye) and deprived (contralateral to sutured eye) SC were sampled with tungsten microelectrodes from 11 to 35 days postnatal. Differences between normal and deprived SC appeared at about 21-22 days in the proportions of oriented directional and indefinite cell types (see Fig. 1). Abnormal receptive fields requiring a bar but not specific for orientation were found in deprived SC beginning at 21 days. No consistent differences were found for other receptive field types, nor was there a difference in the proportion of visually responsive cells.

These results indicate that oriented directional receptive fields in SC will develop in the absence of patterned visual input but this organization will not be maintained in the continued absence of such input. (Supported by grants number EY55551, EY00691, and EY57391 from NEI.)

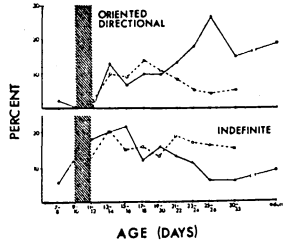

Fig. 1

Fig. 1: Percent of total cells in normal and in deprived SC; open circles: deprived SC; closed triangles: normal SC; closed square: adult data (J. Neurophysiol., 34:148, 1971); open triangle: infant data (Brain Res., 45:67, 1972); shading indicates time of eye opening.

1594 DIENCEPHALIC PROJECTIONS OF THE SUPERIOR COLLICULUS IN THE SQUIRREL MONKEY. A. Frankfurter* and J.K. Harting (SPON: M. Morton-Gibson), Department of Anatomy, University of Wisconsin, Madison, WI 53706

The ascending projections of the superior colliculus were studied in the squirrel monkey using the autoradiographic tracing method. Injections restricted to the superficial layers result in extensive transported protein within the pretectal complex, the posterior nucleus, the inferior pulvinar nucleus, the nucleus limitans, and the ventral lateral geniculate nucleus. Small foci of label are restricted to the intralaminar zones (between layers 1,2,3) of the dorsal lateral geniculate nucleus. Sparse label is also present within the lateral pulvinar nucleus, where it is most prominent in the fibrous region adjacent to the external medullary lamina. Injections involving all layers of the superior colliculus result in transported protein within several additional cell groups. In particular, the intralaminar nuclear complex (i.e., the parafascicular nucleus, the central lateral nucleus, the central superior lateral nucleus, the paracentral nucleus) and the magnocellular division of the medial geniculate nucleusare heavily labeled. Moderate to sparse amounts of label are also present within the subthalamic nucleus, the zona incerta, the substantia nigra, and the reticular nucleus of the thalamus. In several cases, labeled axons can be observed bilaterally within the dorsal and medial aspects of the optic tracts. Such axons can be followed rostrally where they cross within the dorsal portion of the optic chiasm. The terminal field of this pathway is at present unknown. Supported by Grants EY01277 and BM75-00466.

595 CORTICO-PONTINE VISUAL CELLS IN THE CAT. Alan Gibson, George Mower*, James Baker† and Mitchell Glickstein. Dept. of Psychology, Brown Univ., Providence, R.I. 02912.

The cat cortex sends visual information to the cerebellum via a relay in the pontine nuclei. A group of cells in the rostral pons receives an input from the visual cortex, and these pontine cells respond to appropriate visual targets. The purpose of this study was to identify histologically and characterize physiologically the visual cortical cells which send their axon to the pontine nuclei. We first exposed the ventral surface of the pons and located the visually activated area by micro-electrode recording. Horseradish Peroxidase (HRP) was then injected into this pontine visual area. After two days, the cats were perfused, and standard histological techniques were used to react labelled cells. The corticopontine neurons are pyramidal cells in layer 5 and are found in all visual cortical areas.

We also recorded from 25 cells in Area 18 which were driven antidromically by stimulating electrodes placed in the rostral pons. The latency of activation ranged from 1.4 to 6.5 msec. with a median of 2.7 msec.

We conclude that the corticopontine path arises widely from visual cortical areas. The antidromic latencies suggest that the pathway is made up of axons with a relatively broad range of diameters.

1596 LAMINAR DIFFERENCES IN RECEPTIVE FIELD PROPERTIES IN CAT PRIMARY VISUAL CORTEX. Charles D. Gilbert* (SPON: T.N. Wiesel), Dept. of Neurobiology, Harvard Medical School, Boston, Mass. 02115

Cells in different cortical layers differ in their afferents and in the sites to which they project.[1,2] The receptive field (RF) properties of cells in each layer were studied to see how these anatomical distinctions may be refelected functionally. In particular, I wished to test the idea that simple cells receive direct input from the lateral geniculate nucleus (LGNd) by carefully examining their location in relation to the site of termination of geniculate afferents. Microelectrode penetrations were made tangential to the cortical surface, and the position of nearly every unit was marked by a microlesion to identify the layer in which it lay. The RF's studied were located close to the area centralis. Simple cells, defined by mapping with stationary stimuli, were found in the deep part of layer 3, layer 4 and layer 6, never in layer 2 and only rarely in layer 5. Layer 4 consisted almost exclusively of simple cells, whereas the proportion of simple cells in layer 6 was much lower. This distribution closely coincides with that of the terminals from the dorsal layers of the LGNd.[1] The simple cells had distinct properties in each of the layers in which they were found, differing primarily with respect to RF size and shape. The RF's of those in layer 4 were small, and these units often showed a decreased response to long slits. In layer 6 (the origin of the corticogeniculate pathway) the RF's were very long (up to 16^{o}) along the direction of the orientation axis, but narrow (2^{o}-4^{o}), and optimal in response to very long slits. Both simple and complex cells in layer 6 had this shape, and both were activated antidromically from the LGNd. In layer 3 the simple RF's were of intermediate size. The results suggest that there is more than one system of simple and complex cells. 1. Levay and Gilbert, Brain Res. 112 (1976) 2. Gilbert and Kelly, J. Comp. Neur. 163 (1975) 81. (supported by NIH grant # 5T01EY00082)

1597 RESULTS OF PARAMETRIC EXPERIMENTS ON A BLIND VOLUNTEER WITH CHRONICALLY IMPLANTED ELECTRODES. John P. Girvin, Michael G. Mladejovsky* and Wm. H. Dobelle. Dept. of Clin, Neurol. Sci., Univ. and Victoria Hospitals, Univ. of Western Ontario, London, Ontario, Canada N6G 2K3 and Neuroprostheses Program, Inst. Biomed. Eng., Univ. of Utah, Salt Lake City,UT84112

The production of small dots of light in the visual field, i.e. "phosphenes", in response to stimulation of the visual cortex has been known for some time. Chronic implants consisting of 64 platinum electrodes imbedded in Teflon have been placed against the mesial occipital cortex of the right hemisphere (Nature 259:111,1976). A highly compact, 72 pin connector housed in a pyrolytic carbon, transcutaneous pedistal brings the leads through the skin. After more than 8 months (as of May, 1976) there has been no appreciable change in the electrode impedances since their measurement a week post-operatively. Thresholds for capacitively-coupled, balanced-biphasic square waves, 0.25 ms/0.25 ms duration, +/- phases, at 50 Hz (our "standard parameters"), are distributed between 0.9 ma and 4.3 ma. The mean is 1.67 ma $\pm$ 0.11 (SEM), with the distribution skewed toward the lower current range. The threshold for phosphene perception is inversely related to both frequency and pulse duration, At "standard parameters", variation of threshold with train duration is most steep below 10 pulses. No correlation was found with threshold vs the specific area of cortex or with the electrode size. Waveform variations of +/-,-/+,+,-, all capacitively-coupled, cause no appreciable change in threshold in about half of the electrodes. The remaining show minor threshold variations with waveform.

1598 PHOTICALLY ACTIVATED SINGLE UNITS IN VISUAL CORTEX OF MONOCULARLY DEPRIVED CAT. Jay D. Glass, Dept. Pharm., U. Pitts. Sch. Med. Pitts. Pa 15261

Monocular eyelid closure from birth has been described as causing a severe loss in the number of visual cortex neurons that can be driven by stimulation of the deprived eye. In contrast, the author has previously shown a minimal effect of this type of deprivation upon the slow wave, visually evoked response (VER) recorded from one area of visual cortex. Two possible explanations are proposed for these disparate findings. First, the slow wave findings may be unique to the area of cortex studied. Secondly, the single unit activity was evoked by discreet stimuli such as bars and small spots; whereas for the slow wave VER, full field, diffuse stimulation was employed.

To evaluate these possible explanations, a group of cats were monocularly deprived for from 9 to 18 months of age by eyelid closure prior to the normal time of lid separation. The single unit (chloralose anesthesia) and slow wave VERs (chloralose anesthesia and awake) were recorded in response to a diffuse flash. From most of visual cortex the amplitude of the initial positive-negative components of the slow-wave VER evoked from the deprived eye was similar to the VER evoked from the good eye. Certain abnormalities in the waveform of the VER evoked from the deprived eye were indeed present. Diffuse flashes presented to the deprived eye were clearly capable of evoking a strong discharge from single units in visual cortex; 36 of 45 units tested could be driven from either eye. For some units, the response evoked from the deprived eye did differ in latency and discharge pattern from the response evoked from the normal eye. Evidently, monocular eyelid closure does not subsequently prevent neurons in visual cortex from responding to stimulation of the deprived eye. The findings with diffuse photic stimulation are in accordance with the visuobehavioral capabilities of the cat.

1599 LOCAL ADAPTIVE EFFECTS IN RAT RETINAL GANGLION CELLS. Daniel G. Green and Carol M. Cicerone*. Vision Research Laboratory, University of Michigan, Ann Arbor, MI 48109.

Exposure to light reduces visual sensitivity. The neuronal basis for this change in sensitivity is not well understood. Our discovery that light adaptation spreads nonuniformly within the receptive field of a rat ganglion cell shows that the site of visual adaptation must lie prior to the pooling of excitatory responses. A small adapting spot much more effectively decreases the cell's sensitivity to a superimposed test than to test spots in positions far from the adapting locus but still within the receptive field center. In another experiment, a test spot was alternated between two positions within a ganglion cell's receptive field and its intensity was adjusted independently in each position until the cell's response was balanced. When an adapting spot was imaged exclusively onto either position, the balance was upset. The unit became more responsive to the test light when presented to the unadapted position. In a related experiment, a balance was established and then a small intense spot was used to bleach one position, exclusively. During the period of dark adaptation immediately following the termination of the bleaching stimulus, the previously balanced response became unbalanced. These three kinds of experiments show that the effects of light and dark adaptation spread more locally than excitatory effects within the ganglion cell receptive field center. (Supported by PHS grant EY 00379 to D. G. G. and postdoctoral fellowship EY 02161 to C. M. C.)

1600 TECTOFUGAL PROJECTIONS IN GOLDFISH. B.G. Grover* and S.C. Sharma. Dept. of Ophthalmol., N.Y. Medical College, New York, N.Y. 10029.

Efferent projections of the optic tectum in adult goldfish were studied using a modified Fink-Heimer method for staining degenerating axons and terminals. Rostromedial or caudomedial tectal quadrants were ablated unilaterally in ten fish, and survival times of eight to sixteen days were allowed before the brains were processed. Degeneration in the tectal tissue surrounding the ablated area was found in the stratum periventriculare (SP), stratum album centrale(SAC), stratum fibrosum et griseum superficiale(SFGSF), and stratum opticum(SO). Light degeneration was found in the intertectal commissure, and the ipsilateral(and to a lesser extent, the contralateral) torus longitudinalis. In the contralateral tectum, degenerating granules were found in the SP and SAC and were confined to the dorsal tectum. The absence of degeneration in the ventral portion of the contralateral tectum suggests that fibers in the intertectal commissure do not project to that area.

A large group of fibers exits the tectum through the SP. The fibers converge in the lateral tegmentum as they course ventrolaterally, then turn medially to cross in the ansular commissure and descend in the tectobulbar tract. This pattern was found both ipsilaterally and contralaterally to the lesion. Bilateral degeneration was also seen in the commissura transversa, in the tori semicircularis, and in the region of the nucleus isthmus. The ipsilateral nucleus rotundus received input from the tectum. Degeneration was also seen in the posterior commissure.
Supported by USPHS Grant EY-01426 and NSF-GB43506.

1601 IN VITRO UPTAKE OF CHOLINE AND SYNTHESIS OF ACETYLCHOLINE IN THE OPTIC NERVE AND TECTUM OF RANA PIPIENS. E. R. Gruberg* (SPON: L. S. Frishkopf). Research Laboratory of Electronics, M.I.T., Cambridge, MA. 02139.

The optic nerve incubated in vitro in ^{14}C-choline chloride has high affinity uptake of choline (Ch) with saturation at approximately 100 μM. Uptake is inhibited by cooling and by physostigmine and BW284C51. Optic nerve uptake per unit protein is approximately 4 times greater than it is for optic tectum and 25 times greater than the pallium, a putative non-cholinergic forebrain area. A low level of synthesis of acetylcholine (ACh) from ^{14}C-choline chloride is also present. This thus provides further evidence that the optic nerve contains cholinergic fibers. However, that the optic nerve is cholinergic is not sufficient to account for the ACh synthesis observed in the tectum. After cutting the nerve, synthesis of ACh in the deafferented tectal lobe rises relative to the normal lobe. On the contrary, a lesion to the nucleus isthmi reduces the ipsilateral tectal ACh synthesis and acetylcholinesterase activity. Isolating the tectum from rostral or caudal inputs results in no reduction. This suggests that there are two cholinergic inputs to the tectum.

1602 A QUALITATIVE AND QUANTITATIVE STUDY OF GANGLION CELLS OF THE RABBIT RETINA. Mary D. Guthrie* (SPON: M. A. Baker), Richard L. Binggeli, George P. Moore and Peter F. Cummings* (SPON: M. A. Baker). Dept. of Anatomy, Univ. of Southern California Schl. Med., Los Angeles, CA. 90033.

Pigmented New Zealand rabbits were used to study properties of ganglion cells of the retina by introducing a low impedence microelectrode into the optic nerve and recording action potentials following 35 kinds of quantitative and 6 kinds of qualitative tests. Each unit was assigned properties as follows: Receptive field, ½-1°, 1-3°, or 4° or more; Preferred target size, 1°, 3°, 4° or more; Preferred target velocity, 15°, 30°, or 45°/sec., Spontaneous Activity and Directionality were noted. 69.6% of the units had small receptive fields and most of these preferred medium size targets with fast velocity, had no spontaneous activity and were non-directional. 8.9% of the units had medium size receptive fields; most of preferred large targets with fast velocity, had no spontaneous activity and were non-directional. 21.4% of the units had large receptive fields; most of these preferred medium size targets moving at medium to fast speeds, and were most apt to show spontaneous activity but no directionality. Of the 23% that were directional, most were apt to have a small receptive field. Only one unit out of the 77 had both spontaneous activity and directionality. Unit responses to 5 color and 5 spot size flashes at various time intervals were collected and quantified. The means of the number of impulses following ON-OFF color stimulation are very similar with the exception of RED FLASH. Calculations of ON-OFF means and frequency distributions show a tendency toward a homogeneous population of units. With these stimuli the mean OFF response was greater than the mean ON response in every case.

1603 SUSTAINED SEARCH FOR LINEAR Y-CELLS. D. I. HAMASAKI* and VESNA G. SUTIJA. Bascom Palmer Eye Institute, Miami, Fla.

The responses of retinal ganglion cells were examined with a contrast-reversal type of stimulus in adult cats. At intermediate levels of adaptation and stimulus contrast, units could be segregated into those showing linear spatial summation and a null position within the receptive field (X-cells), and those showing non-linear spatial summation and in which a null position could not be found (Y-cells).

With the stimulus at the null position, X-cells could be made to respond in a non-linear way by changing either the contrast or the size of the stimulus, or by changing the state of adaptation. For the Y-cells, it was not possible to find stimulus conditions which made the cells respond in a linear way. However, at very low levels of adaptation and low stimulus contrast, the responses of Y-cells were more like the X-cells.

The linearity or non-linearity of spatial summation results from the interaction of the center and surround mechanisms of the receptive field. The responses will be linear only when these mechanisms are critically balanced. Thus any manipulation of the stimulus which favors one mechanism over the other will result in a non-linear response.

1604 LAMINAR DIFFERENCES IN SOME UNCROSSED PROJECTIONS OF THE SUPERIOR COLLICULUS TO THE MIDBRAIN. Craig K. Henkel and Stephen B. Edwards. Dept. Anat., Sch. Med., Univ. Va., Charlottesville, Va. 22901.

The uncrossed projections of the superior colliculus to the central and caudal midbrain in the cat were traced by autoradiography in transverse and horizontal sections following stereotaxically placed injections of ^{3}H leucine. The uncrossed projections arise largely from the stratum griseum intermedium and the stratum griseum profundum. The distribution of the projections of these two layers is, however, strikingly different. The stratum griseum profundum projects heavily to a restricted tegmental zone along the medial border of the medial lemniscus and to cell bridges located within the medial lemniscus itself. This area contains densely arranged, dark-staining neurons and appears to be part of the pedunculopontine tegmental nucleus. In some cases the projection from the stratum griseum profundum extends caudally into the pontine reticular formation. In sharp contrast, the stratum griseum intermedium projects to a smaller tegmental zone dorsal to the previous and adjacent to the parabigeminal nucleus. Axons reach these zones by leaving the colliculus in stratum album intermedium and then coursing ventrally and caudally along the medial surface of the brachium of the inferior colliculus. The stratum griseum superficiale and possibly the stratum opticum, on the other hand, project only to the parabigeminal nucleus. Together these uncrossed connections constitute one of the most massive efferent systems of the superior colliculus.

1605 RESPONSES OF CAT VISUAL CORTICAL CELLS TO DRIFTING SINUSOIDAL PATTERNS: CORRELATION OF SPATIAL AND TEMPORAL TUNING. R.A.Holub and M.Morton Gibson. Depts. Neurophysiol. and Ophthalmol., U. Wis., Madison, WI. 53706.

We are employing a Maxwellian optical system in a quantitative study of the responses of single cortical cells to images whose focus on the fundus is assured by indirect ophthalmoscopy through the system's field lens. We have stimulated primarily with optimally-oriented, moving, elongate sinusoidal gratings, although more conventional stimuli (slits and borders) are systematically employed. Movement of the gratings results in a temporal modulation of the luminance over the receptive field. Almost all cells studied are within 5^{o} of the center of gaze in visual areas I and II of lightly anesthetized, paralyzed cats. A classic LINC digitizes times of spike occurrence on-line. The sample currently includes some 60 useful cells, about 30 of which have been studied in excess of 4 hrs., and indicates that 1) with very few exceptions, cortical cells respond differentially to regions of the spectrum of sinusoidal spatial frequencies to which the cat is sensitive (.1 to 3.5 cycles per degree of visual angle). 2) 70% or more do so with enhanced discharge in some range of spatial frequencies, 15% are inhibited (below their background rate) in some region and 10% have 2 peaks of enhancement in the spatial spectrum. The relations of these broad categories to the accepted varieties of receptive fields will be outlined. 3) The best temporal and/or spatial frequencies are not necessarily the same in the two directions of movement. 4) There is a negative correlation between the best temporal frequency (at constant cyc/deg) and the best spatial frequency (at constant Hz) although the scatter of points is considerable.

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1606 RECEPTIVE FIELD PROPERTIES OF THE VENTRAL LATERAL GENICULATE NUCLEUS OF THE CAT. C. Hughes and S. Ater. Department of Neurology, Washington University, St. Louis, Mo. 63110.

As the initial part of a study of the functional properties of the ventral lateral geniculate nucleus in the cat, the visual receptive fields of 130 neurons were studied. With the animals under light barbiturate anesthesia and paralyzed with a continuous infusion of flaxedil, single units were studied with tungsten electrodes and mapped on a tangent screen. The nucleus was approached utilizing detailed retinotopic maps of the dorsal lateral geniculate nucleus prepared by others and electrode tracts and positions verified histologically. The receptive fields were arbitrarily divided into 5 subtypes; uniform or concentric large (6-40°) fields (40%), uniform or concentric small (1-2°) fields (29%), spontaneously firing units poorly responsive or unresponsive to visual stimuli (16%), large receptive fields covering the contralateral visual field or a quadrant of the visual field (9%), and color coded units (6%). The majority of the large uniform-concentric fields responded to stationary stimuli preferentially but sluggishly although 1/5 of them did respond well to movement. Of the latter group, 3 units were directionally selective. The small uniform-concentric units responded much more briskly and resembled those found in the dorsal lateral geniculate nucleus. Color coded units were either opponent (blue-on, green or red off) or responded to blue better than to white, green, or red. Only 15% of the units were driven binocularly, most of the rest being activated by the contralateral eye only and 2 units resembled luminance detectors in that their response changed linearly through a wide range of background light intensity. Receptive field properties of this small nucleus show considerable diversity suggesting multiple functions.

1607 FUNCTIONAL CONSEQUENCES OF BINOCULAR INNERVATION OF A SINGLE OPTIC TECTUM IN FROGS. David Ingle, McLean Hospital, Belmont-MA 02178

In frogs with one optic tectum removed, cut optic fibers regenerate to the remaining ipsilateral tectum, forming functional connections in addition to the normal contralateral connections. Our study of single neurons in such frogs with (abnormal) binocular receptive fields was designed to reveal central visual processes (facilitatory or inhibitory) free of intraretinal influences. This was achieved by presenting a moving dark spot first via one eye's receptive field (RF) and secondly to the opposite eye's RF. From a number of experiments on isolated tectal cells showed that for units with marked habituation properties, the habituation transferred interocularly.

While most tectal neurons had symmetrical RF's with similar response properties, a few were distinctly asummetrical. First, some cells showed prolonged discharge to a stopped spot via one eye, but only an intense brief response via the other. A second and more dramatic effect involved total suppression of response via the ipsilateral RF until the contralateral eye was covered. Then a vigorous response could be elicited. These two independent phemonema suggest that both eyes could innervate the recorded cell while unequally connected to other modulating neurons. Whether the modulating systems which produce asymmetrical behavior are intrinsic to the tectum is also under investigation.

1608 MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONES WITH CALLOSAL AXON IN CAT'S VISUAL CORTEX. G. M. Innocenti* and L. Fiore* (SPON: M. T. Shipley). Institut d'Anatomie, Université de Lausanne, 1011 Lausanne, Switzerland.

Location, morphology and functional properties of neurones projecting from primary visual areas into the callosum were studied in adult cats, using retrograde transport of horseradish peroxidase (HRP) and single unit recording. HRP injections in a roughly 5 mm wide strip straddling the 17-18 border resulted in retrograde labelling of neurones located in contralateral areas 17 and 18. The HRP positive neurones were spread over a region located around the representation of the vertical meridian of the visual field. They were most numerous in the locus for area centralis. In both areas 17 and 18 the main contribution to the callosum is from layer III pyramids of all sizes and from layer IV stellate cells.

In acute electrophysiological experiments neurones with callosal axon were identified by their antidromic response (1.4-2.8 msec latency;) to the electrical stimulation of contralateral cortex. From the functional point of view they constitute a heterogeneous group, that contains units with "simple", "complex", and "hypercomplex" properties. Apparently, the only physiological property common to neurones projecting into the callosum is the rather small size of their receptive field (0.65-1.5°). The distribution in depth of antidromically activated units corresponds to that of HRP labelled cell bodies.

1609 THE EFFECT OF BILATERAL NEOMATAL EYELID SUTURE ON VISUAL DISCRIMINATION IN THE RAT. John A. Jane and Shelia Scoville*. Dept. Neurosurgery, Univ. of Virginia School of Medicine, Charlottesville, VA 22901.

The effects of visual deprivation on function have been studied extensively by physiological methods. On the other hand, the behavioral effects that follow eyelid suture are less well known. A series of hooded rats were prepared with neonatal eyelid suture and subsequent release at maturity. No deficits in the descrimination of gross flux, stripe orientation or inverted triangles were found. However, deprived animals failed to descriminate triangles contained within an annulus. These findings are similar to the effects of destruction of the superficial layers of visual cortex which removes the important intracortical connection from supragranular to infragranular layers. The similarity in behavioral results suggest the possibility that this intracortical connection might be affected by neonatal pattern deprivation.

1610 THALAMIC INPUTS TO THE RABBIT VISUAL CORTEX: IDENTIFICATION AND ORGANIZATION USING HORSERADISH PEROXIDASE (HRP) A.N. Karamanlidis* and R.A. Giolli, Dept. of Anat., Calif. College of Med., UCI, Irvine, CA. 92717.

Preliminary data are reported on the thalamic inputs to the rabbit visual cortical area I (VI) of Thompson et al. (J. Neurophysiol., 13: 277-288, 1950) which correspond to the striate cortex of M. Rose (J. Psychol. Neur., 43: 353-440, 1931). Thusfar, injections of HRP into discrete regions of the VI have revealed (i) labeled cells in the ipsilateral dorsal lateral geniculate nucleus (LGd), the pulvinar and the posterior thalamic nucleus (delineated according to J.E. Rose, J. Comp. Neur., 77: 469-523, 1942) and (ii) a reciprocity between the LGd and the striate cortex via geniculocortical and corticogeniculate projections as well as retinotopic organization in the geniculocortical projection, corresponding to that demonstrated for the corticogeniculate projection by Giolli and Guthrie (J. Comp. Neur., 142: 351-376, 1971). Studies are currently under way to show the organization of the input to VI from the pulvinar and the posterior thalamic nucleus. (Supported by a Fogarty International Fellowship to A.N. Karamanlidis and by NIH grant 5R01 EY00607).

1611 CATECHOLAMINERGIC CONTROL OF NEURAL PLASTICITY IN KITTEN VISUAL CORTEX DURING THE CRITICAL PERIOD. Takuji Kasamatsu and John D. Pettigrew. Div. of Biology, California Inst. of Technology, Pasadena, CA 91125.

Previously we observed the failure of ocular dominance shift in monocularly deprived kittens whose brain catecholamines had been depleted by repeated injections of 6-hydroxydopamine (6-OHDA) (ARVO meeting, 1976). To clarify this phenomenon, we have carried out studies of the dose-response relationship of the time course of the drug's action and of 6-OHDA's effect in a number of control situations.

A cumulative dose of 10 mg intraventricularly 6-OHDA appears to be necessary to produce sufficient catecholamine depletion for a complete suppression of any effect of eye occlusion on the ocular dominance of single cortical units. This effect lasts some weeks after drug treatment stops. Rerecordings from the same monocularly-deprived kitten at various times after cessation of drug treatment show a gradual recovery of the kitten's sensitivity to monocular occlusion.

An indoleamine-depleting neurotoxin, 5,7-dihydroxytryptamine, appeared to produce an enhancement of the ocular dominance shift.

6-OHDA appears to have little direct effect on cortical cells' properties since we observed no significant changes in ocular dominance distributions after giving large doses to kittens with previous monocular deprivation and to normal kittens and adults. We conclude that 6-OHDA specifically reduces plasticity by reducing catecholamines.

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1612 FUNCTIONAL ORGANIZATION OF THE RABBIT STRIATE CORTEX. Amy Schick Kelly* and Patricia C. Fox (Spon., L.F. Eng). Dept/Neurology, Stanford Univ. Sch. Med., Stanford, CA 94305.

A detailed picture has emerged in recent years of the functional organization of striate cortex in binocular mammals, most notably, in monkeys and cats. It is of interest now to ask whether any features of this organization are present in the striate cortex of mammals which have little or no binocular overlap. Such animals have almost entirely crossed retinogeniculate projections, and will necessarily lack the ocular dominance columns which are the most salient feature of binocular striate cortex. To approach this question, we have studied electrophysiologically the organization of the striate cortex in adult dutch-belted rabbits. Long tangential penetrations were made through the visual cortex with tungsten microelectrodes. Receptive fields were assayed for single units or small groups of units every 25mμ, or whenever a change in recorded activity was observed. We found that periodic discontinuities or shifts in receptive field position occurred as the electrode was advanced along a track. These shifts were separated by an average track distance of 1/2 mm, and tended to be abrupt, often occurring within one 25mμ step. Between the shifts, the receptive fields were extensively overlapped; envelopes drawn around such groups of fields averaged 10° in diameter. Envelopes drawn around successive groups of fields overlapped only slightly. The groups of fields appeared to include a random distribution of functional types, both oriented and non-oriented. Evidence for a sequential progression of orientation preference was obtained in only a few tracks, probably due to the normal high percentage of un-oriented cells (70%). These results indicate that the rabbit striate cortex may be organized into columns representing spatial subdivisions of the visual field, although it lacks the organizational constraint of binocular input. (Supported by grants number EY57391, EY55551 and EY00691 from the NEI, NIH.)

1613 THE INVOLVEMENT OF GAMMA-AMINOBUTYRIC ACID (GABA) AND GLYCINE IN THE ORGANIZATION OF CAT RETINAL GANGLION CELL RECEPTIVE FIELDS. Albert W. Kirby. Ophthal. Dept., Kresge Eye Institute, Wayne State University, Detroit, Michigan 48201.

It was reported last year at this meeting that GABA is involved in receptive field organization of Y- but not X-type cat retinal ganglion cells. Additionally, it was suggested that the importance of GABA to Y-cells was dependant upon the luminance of the stimulating light. This was interpreted to suggest a GABA-containing amacrine intermediate in the rod pathway which is not involved in the transmission of signals originating in the cones. Additional results obtained extra-cellularly from single units in the optic tract of anesthetized cats after administration of picrotoxin or bicuculline (GABA antagonists) will be presented.

Glycine, another putative retinal neurotransmitter, is also associated with amacrine cells which are presumably a separate sub-population from those containing GABA. For this reason strychnine, a glycine antagonist, was administered intravenously to cats while studying the receptive field properties of retinal ganglion cells. While strychnine often elevates the maintained firing rate of Y-cells with no consistent change in center-surround balance, the effect on X-cells closely parallels that of GABA antagonists on Y-cells, but is somewhat less consistent.

Before defining the circuitry and pharmacology of the inner plexiform layer with any certainty, it is first necessary to localize the putative transmitters to a particular morphological group of amacrine cells; secondly to determine whether or not the transmitters are hyper- or depolarizing the postsynaptic neuron. Being fully aware of these limitations, the results will be discussed in terms of receptive field organization and pharmacological differences of cat retinal ganglion cells.

1614 THE EFFECTS OF MONOCULAR DEPRIVATION ON THE MEDIAL INTERLAMINAR NUCLEUS OF CATS. K. E. Kratz*, S. V. Webb, and S. Murray Sherman. (SPON: G.R. Hanna). Dept. of Physiol., Univ. of Virginia Medical School, Charlottesville, Virginia 22901

We used extracellular single-unit recording techniques to investigate single cells of the medial interlaminar nucleus (MIN; a division of the LGNd) in normal adult cats and adult cats that were monocularly lid-sutured (MD) from birth. We found, in agreement with Guillery's[1] anatomical findings, that a small central portion of MIN receives input from the ipsilateral eye while a much larger crescent-shaped portion surrounding the central area receives input from the contralateral eye. Also in agreement with previous reports[2,3] we found that MIN is composed of cells having Y-type receptive field characteristics and short latencies to electrical stimulation of the optic chiasm. In addition, cells in MIN show no indication of the binocular inhibitory interactions found in cells of lamLGNd (i.e., laminae A, A1, etc.). In normal cats, responsive cells were always encountered in both the ipsilaterally and contralaterally represented portions of MIN. In contrast, in MD cats relatively few cells were encountered in that part of MIN receiving input from the deprived eye, while normal numbers of cells were found in the non-deprived portion of MIN. Furthermore, many of the MIN cells that were driven by the deprived eye were abnormal in either their receptive field size, receptive field characteristics, or latency to optic chiasm stimulation. These data indicate that the Y-cells in MIN, like the Y-cells in lamLGNd, are sensitive to the effects of monocular lid-suture.

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1. R.W. Guillery, *J. Comp. Neurol.* 138: 339-368 (1970).
2. R. Mason, *Exp. Brain Res.* 22: 327-329 (1975).
3. L.A. Palmer, A.C. Rosenquist, R. Tusa, *Neuroscience Abstracts* (1975).

1615 DEFICITS IN VISUAL DISCRIMINATION PERFORMANCE AND EYE MOVEMENTS FOLLOWING SUPERIOR COLLICULUS ABLATIONS IN RHESUS MONKEYS. Daniel Kurtz* and Charles M. Butter. Dept. Psychol., University of Michigan, Ann Arbor, Mich. 48109.

Monkeys with their heads immobilized were trained to discriminate between two 0.2° spots differing in color and spatially separated by varying distances from the two response panels while their eye positions were monitored by electro-oculograms. The two response panels were located 8° to either side of center gaze, and on each trial the discriminative cues were presented either 8°, 15°, 21°, 27°, or 32° to the left and right of center gaze. During training, discrimination performance was inversely related to the degree of stimulus-response separation, and correct performance was positively correlated with fixation of the correct cue. Following bilateral ablation of the superior colliculus, discrimination performance and fixation of the correct cue were reduced compared to pre-operative levels only when the stimuli were separated from the response panels. These deficits were present several weeks following surgery and were more pronounced when the discriminative stimuli were located 27° and 32° from center gaze. Following colliculus surgery, the monkeys were also reluctant to fixate bits of fruit presented 27° or 32° from center gaze, although they continued to reach for them accurately. Monkeys with sham control lesions did not show these performance and eye fixation deficits. These results suggest that, under the conditions employed in this experiment, monkeys with superior colliculus lesions are deficient in foveating peripheral visual stimuli by ocular movements.

1616 COLLICULAR AFFERENTS AND PATTERNS OF CELLS IN THE CAT SUPERIOR COLLICULUS. Thomas P. Langer* (SPON: P. Coates). Dept. Biological Structure, Univ. Wash. Sch. Med., Seattle, Washington 98195 USA.

A morphometric Golgi study and degeneration and autoradiographic studies of the retinal and visual cortical projections were done. The crossed retinal projection was primarily restricted to the superficial portion of the stratum griseum superficiale (II 1 of Kanaseki and Sprague) while the ipsilateral, uncrossed, retinal projection was to the fibrous portion of stratum zonale and the deep portion of stratum griseum superficiale (I 1 and II 2) with occasional patches in II 1. Both projections were irregularly clustered in both horizontal and vertical directions. The contralateral retinal projection has a local graininess seen as an irregular clustering of autoradiographic grains, Fink-Heimer granules, or neurofibrillar rings (clusters 10-20μ across), a larger scale clumping of all three into regions of greater density (100μ deep by 500μ wide), and consistent regional differences (denser peripherally, less dense in area centralis representation). The ipsilateral retinal projection shows similar organization, but different patterns. The primary visual cortical projection was distributed into the same laminae as the ipsilateral retinal projection, but is considerably denser than either retinal projection, extends to the anterior margin of the colliculus, and shows no obvious clustering.

The morphometric Golgi study demonstrated a consistent, complex distribution of a limited number of types. The general types seen and their relative positions accorded with the pattern seen in rat (Langer and Lund, '74, J. Comp. Neurol. 156: 405; Langer, '75, Anat. Rec. 181: 404), but there was a greater diversity of types in a less distinct pattern. The patterns of cells accorded well with the afferent distributions, less well with the classic strata of the superior colliculus.
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1617 RETINOGENICULATE PROJECTIONS OF INBRED MICE: WITHIN- AND BETWEEN-STRAIN COMPARISONS. Larry Leach* and Irwin S. Westenberg*, V.A. Hosp., Phoenix, Az., U.S.A., 85012. (SPON: E. Davis).

In comparisons of coisogenic B10.D2/nSn albino (c^{4J}/c^{4J}) vs. black ($+/c^{4J}$) mice, reduced uncrossed retinogeniculate projections (RGP) were observed. However, differences in crossed RGP were more difficult to assess. The crossed RGP of black B10.D2/nSn (+/+, n=6 and $+/c^{4J}$, n=5) mice did not appear to have a clearly defined "pocket" of reduced argyrophilia, as has been described for other rodents. In order to determine if this lack of a pocket was unique to the B10.D2/nSn strain, we examined the crossed RGP of two very closely related strains (B10.D2/oSn, n=6 and C57BL/10Sn, n=6), one distantly related strain (C57BL/6J, n=4), and one strain that had been crossed once with distant ancestors of the B10.D2/nSn strain (DBA/2J, n=6). Six or seven days following removal of the right eyes, the mice were perfused; horizontal brain sections were stained for degenerating optic tract processes. In some cases a clearly defined pocket was observed. More often, it was difficult to define a pocket, although there appeared to be a reduced input to the region receiving uncrossed RGP. The discriminability of a pocket was not a function of the presence or absence of a "patch" of uncrossed input, because clearly defined patches were observed in all strains. Variables that influence the detection of a pocket include strain, plane of section, thickness of the section, stain, microscope illumination (e.g. bright- vs. dark-field), depth of field of the microscope objective, and photomicrographic emulsion (e.g. high- vs. low-contrast).

1618 RESPONSE CHARACTERISTICS OF MONOCULAR AND OF BINOCULAR NEURONS IN THE VISUAL CORTEX OF PATTERN DEPRIVED CATS. Audie Gene Leventhal* and Helmut V.B. Hirsch. Center for Neurobiology, SUNYA, Albany, NY 12222.

The response characteristics of over 300 cortical neurons in a group of normal adult cats and over 200 cortical neurons in a group of cats deprived from birth to 10-12 months of age were investigated.

Most cortical neurons (80%) which had large receptive fields or which responded to rapid stimulus motion were activated binocularly in normal adult cats. This proportion was unaffected by deprivation of patterned visual stimulation: 73% of the cortical neurons of this type were activated binocularly in pattern deprived cats.

A majority of cortical cells (88%) which had small receptive fields and which responded only to slow stimulus motion were activated binocularly in normal adult cats. This proportion was reduced in animals deprived from birth of patterned visual stimulation: 70% of the neurons of this type were activated monocularly in pattern deprived cats. Most of these monocularly activated cells were responsive only to stimulation of the contralateral eye.

These results suggest that certain neurons in the cat's visual cortex do not require early visual stimulation for the development of binocularity while the binocularity of other cells is dependent upon early visual experience for development or for maintenance.

1619 RETINAL MÜLLER CELL INDUCTIVE PROPERTIES? Jeffrey Levett* and Jim McAvinn* (SPON: J.A. Michael). Rush-Presbyterian-St. Luke's Med. Ctr. Chgo, Ill. 60612

At certain levels of adaptation the b-wave in the retinal response to light shows oscillatory behaviour (Auerbach, Doc. Ophthal. 22, 1, 1967) Ganzfeld studies in the intact frog reveal the same phenomenon. Appearance of the E-wave takes place under intermediate levels of adapting luminance with an intense flash stimulus. Generation of the b- and E-wave can be modelled by a parallel RLC network suddenly driven by an appropriate forcing function which is a light induced K+ current that depolarises the Müller cell membrane and its time constant is a function of adaptation. The resistance is also a function of adaptation. An inductive element L enables the model to generate the oscillatory behaviour incorporating the E-wave. To the best of our knowledge an analog has not previously been suggested for visual systems. Cole's work (J. Gen. Physiol. 24, 771, 1941) suggests possibility of an inductive element in neural system due to presence of K+ ions. Müller cells, responsible for the b-wave (Miller and Dowling, J. Neurophysiol. 36, 28, 1970) and most probably the E-wave in our studies are surrounded by K+ ions. Hodgkin (Biol. Res. 26, 339, 1951) considered as did Cole that inductive reactance and oscillatory phenomena might have a common origin resulting from K+ permeability changes. That the b-wave and E-wave are part of the same mechanism is suggested by temperature experiments. It appears that the E-wave is not part of a delayed off-effect.

1620 PROPERTIES OF MONKEY RETINAL GANGLION CELLS AND THEIR TECTAL PROJECTIONS. Joseph G. Malpeli and Peter H. Schiller*. Dept. Psych., MIT, Cambridge, MA 02139.

The response properties of 1025 retinal ganglion cells were assessed quantitatively using intra-ocular recording methods. Their projection sites and conduction velocities were determined by antidromic activation from the superior colliculus, lateral geniculate nucleus, and optic chiasm.

Most cells had concentrically arranged receptive fields and could be classified as either color-opponent or broad-band. Color-opponent cells have relatively slow conducting axons and fire in a sustained manner to stationary stimuli. Broad-band concentric cells have fast conducting axons and respond transiently to stationary stimuli. The ratio of color-opponent cells to broad-band cells is at least 3 to 1. This ratio does not vary with distance from the central fovea within the range of our sample (1°-20° eccentricity). In addition, we recorded from a small group of non-concentric ganglion cells. Although their response properties indicate that these are a heterogenous group, all had slowly conducting axons, very low spontaneous activity, and broad-band color sensitivity.

The superior colliculus receives input from both concentric and non-concentric broad-band cells, but not from color-opponent cells. When sampling biases are taken into account our results suggest that the non-concentric cells are the major retinal input to the superior colliculus.

1621 RELATIVE MOVEMENT OF VISUAL PATTERNS: THE RESPONSES OF SINGLE CELLS IN CAT SUPERIOR COLLICULUS: G. Mandl, Aviation Med. Res. Unit, Dept. of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

Behavioural experiments indicate that the velocity of a moving visual pattern is judged more reliably from its changing spatio-temporal relation to other patterns (relative velocity), rather than from the velocity of its image on the retina (absolute velocity). The present experiments were undertaken to determine the presence, and the characteristics, of collicular visual cells specifically responsive to patterns moving relative to each other. Methods. Unanaesthetized, paralyzed, pretrigeminal cat preparations were used. Visual stimuli consisted of small "target" patterns (lines, dots, not exceeding 1°), superimposed upon "background" patterns (square wave gratings, 0.5-1 cycle/°). Stimulation was accomplished with various combinations of "target" and "background" pattern velocities. Unit discharges from the right (contralateral) superior colliculus were recorded extracellularly. Results. (1) With 35% of recorded visual cells, the response to one moving pattern could be systematically modified by the presence of other (moving or stationary) patterns in the receptive field. Such alterations were not the result of changes in mean luminance. (2) Within the range of velocities tested (1-240°/sec), a given unit responded optimally whenever the relative velocity between two simultaneously moving patterns attained a unique value characteristic for that unit. By using a number of velocity ratios, a "family" of velocity tuning curves, relating unit response to relative pattern velocity, could be generated. Each of the "member" curves had its peak at the unit's characteristic relative velocity value.

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1622 FURTHER EVIDENCE ON DISSOCIATION OF VISUAL DEFICITS FOLLOWING PARTIAL INFERIOR TEMPORAL LESIONS IN MONKEYS. Frederick J. Manning and Mortimer Mishkin. WRAIR, Washington, D.C. 20012 and NIH, Bethesda Md, 20014

Severe impairment on a delayed matching-from-sample task was found after lesions of the anterior but not of the posterior part of the inferior temporal cortex (Mishkin & Oubre, NA: 1976). The finding is consonant with earlier suggestions that anterior temporal lesions yield visual memory deficits that are dissociable from the visual discriminative deficits produced by posterior temporal lesions. In the delayed matching study, however, reliable impairment was found only with anterior temporal removals that were larger than in any previous study. They included the rostral two-thirds of the inferior temporal convexity (area TE) rather than just the middle third, while the posterior temporal removals were limited, as in earlier studies, to the caudal third of the convexity (area TEO). In order to determine whether the dissociation of deficits found earlier could be obtained despite the increased size of the anterior removal, monkeys with TE and TEO lesions were compared on automated versions of both delayed matching with a pair of grossly dissimilar stimuli and simultaneous discrimination learning with pairs of highly similar patterns. The TE lesion produced the greater effect on delayed matching, yet the smaller TEO lesion still had the greater effect on pattern discrimination learning. The results thus support the original proposal that the posterior part of the inferior temporal cortex participates mainly in visual discriminative functions while the anterior part contributes primarily to visual memory processes.

1623 RETINOTECTAL INPUT TO MONKEY SUPERIOR COLLICULUS.
R.T. Marrocco*and R. Li* (SPON: C.B. Kimmel). Dept. of Psychol., Univ. of Oregon, Eugene, OR. 97403.

Receptive field (RF) properties of cells in the paralyzed macaque superior colliculus were studied with stationary and moving visual stimuli. The conduction velocity (CV) of the retinal axonal input to these cells was determined by shocking optic chiasm.

Most cells responded best to stimuli moving in any direction. Many superficial cells discharged only to the leading and trailing edges of moving borders; deep layer cells discharged to each border and the even illumination in between. Superficial cells were tuned to a wide range of velocities; some followed objects moving at more than 1000 °/s. In contrast, deep cells were narrowly tuned and rarely followed movements above 60 °/s. Results of chromatic adaptation studies suggested that all cells were non-opponent.

Shock latencies were unimodally distributed with a mean of 7.8 ± 1.6 ms (CV = 3.4 m/s). They varied with a cell's retinal eccentricity, RF area, ocular dominance group, type of response to moving borders, and depth. Superficial cells had short latencies, deep cells had longer latencies. There was a positive correlation between a cell's visual velocity preference and its CV. Latencies were not correlated with the presence/absence of directional selectivity. Results support the hypothesis that CV groupings are related to differences in RF types between, rather than within layers of SC.

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1624 ELECTROPHYSIOLOGICAL ANALYSIS OF THE RETINOTECTAL PROJECTION IN GOLDFISH FOLLOWING UNCROSSING OF SELECTED OPTIC RADIATION FIBERS.
Ronald L. Meyer* (SPON: R. W. Sperry). Biology Division, California Institute of Technology, Pasadena, CA. 91125.

Fascicles of optic fibers supplying dorsocaudal optic tectum were teased free of surrounding tectum to the anterior tectal pole near their exit from the medial brachium of optic tract and were deflected into a small incision at the rostromedial edge of the contralateral "host" tectum, the entire optic fiber supply of which was sectioned at the same time either in the orbit or at rostral tectum. After 3-10 months the projection maps for the regenerated "transplanted" and "host" fibers were determined by eye-in-water electrophysiological mapping. The "transplanted" fibers appeared to terminate only in dorsocaudal tectum with good relative ordering but often terminated on neighboring rather than strictly appropriate loci. The "host" fibers projected correctly and retinotopically over most of the tectum but within the dorsocaudal target area fibers often terminated at neighboring inappropriate loci. These displaced "host" fibers in general tended to terminate more rostrally than normal on dorsal tectum creating a kind of topographic compression. Thus, optic fibers demonstrated a tendency toward equidensity innervation and toward topographic ordering with some local plasticity with respect to tectal loci. These plasticities cannot be a consequence of postulated field-type regulative changes of cytochemical properties since both retinal and tectal integrity were preserved in these experiments. The results can be explained by interfiber competition for tectal space and by selective chemoaffinity interactions not only between optic fibers and tectal cells but also between optic fibers themselves. (Supported by the Caltech Hixon Fund and NIMH grant MH03372 to R. W. Sperry.)

1625 DISSOCIATION OF DEFICITS ON VISUAL MEMORY TASKS AFTER INFERIOR TEMPORAL AND AMYGDALA LESIONS IN MONKEYS.
Mortimer Mishkin and John L. Oubre*. NIMH, Bethesda, Md. 20014

A study in normal monkeys (Mishkin & Delacour, JEP-ABP 1: 326, 1975) demonstrated that visual memory can be separated into partly independent components by use of variations of delayed matching. The standard test, delayed matching with a single pair of repeatedly presented stimuli, taxes memory in two ways; it requires both identification of the more recently presented of two highly familiar stimuli and association of that abstraction (more recent stimulus) with the reward. One variation, delayed nonmatching with trial-unique stimuli, taxes stimulus recognition instead, eliminating or greatly reducing the burden on the other more complex components of visual memory that are presumably built upon stimulus recognition. In an attempt to relate these components differentially to neocortical and limbic mechanisms, the two tests were presented for relearning to separate groups of monkeys with bilateral removals of either the amygdala, the hippocampus, or the anterior or posterior part of the inferior temporal cortex (areas TE and TEO, respectively). On the standard test, both the amygdala and area TE lesions produced severe impairment, while the other lesions had little if any effect. On the test variation, however, though the TE lesion again produced severe impairment, the amygdala lesion, like the others, produced none. The results suggest that area TE is important for visual recognition, whereas the amygdala contributes only to later stages of memory. The results fit a neural model (Jones & Mishkin, EN 36: 362, 1972) that views the inferior temporal cortex as a link in a functional chain leading from the primary visual area to the limbic system.

1626 LATERAL GENICULATE AND SUPERIOR COLLICULUS CELL ACTIVITY STUDIED BY SIMULTANEOUS RECORDINGS PRIOR TO AND FOLLOWING CORTICAL DEPRESSION.
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The activity of Geniculate (L.G.N.) and Collicular (S.C.) neurons was simultaneously recorded in nembutal anesthetized rabbits, with two tungsten microelectrodes. The unitary activity was evoked following stationary ON and OFF stimuli and flicker presentation. Receptive fields of some paired units were characterized. The Visual Cortex and Optic Nerve were electrically stimulated. Responses of Geniculate neurons to the latter permitted the differentiation of relay cells and interneurons which responded with a burst to a single shock. The results revealed that some Geniculate and Collicular cells presented firing patterns which were opposite in phase, that is periods of high activity in one unit coincided with a reduced rate or a complete absence of spike in the complementary cell. This alternating activity was observed for up to 800 msec. following the initial stimulus. Such fluctuations were also evoked by electrical stimulation of the visual cortex. In order to study the influence of the visual cortex upon geniculate and collicular cell activity, cortical function was abolished by applying 3M KCL over its surface: this resulted in a considerable disruption of the time course of the control firing pattern in both L.G.N. and S.C. Specifically, in the L.G.N. pre-excitatory inhibitory periods were abolished while post-excitatory inhibitory periods were enhanced in intensity and duration. The Collicular neurons reacted to the same treatment with a reduction of the firing rate of the after-discharge. These results suggest that the Visual Cortex mediates these alternating types of activity.
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1627 MEMBRANE STRUCTURE OF HORIZONTAL CELL CONTACTS IN THE OUTER PLEXIFORM LAYER OF THE CATFISH RETINA. Andre R. Nagy* and Ken-Ichi Naka. Jules Stein Eye Institute, UCLA Sch. Med., Los Angeles, CA 90024 and California Institute of Technology, Pasadena, CA 91125.

Freeze-fracture analysis, correlated with serial thin sectioning of conventionally prepared and Golgi-impregnated retinas of the catfish (Ictalurus punctatus), permitted evaluation of connections and membrane specializations at the site of contact. Dendrites of both external and intermediate horizontal cells contact receptor terminals by symmetrical bilobulated invaginations below the apex of the synaptic ridge. These large lobular processes are easily distinguishable from the thin branches of invaginated bipolar dendrites.

Freeze-fracture of the receptor terminals reveals intramembrane aggregates of particles of the cytoplasmic membrane-half (A face) of the synaptic ridge and a paucity of particle organization away from the ridge. The external membrane-half (B face) of the receptor terminal is relatively unspecialized. Membranes of horizontal cell processes exhibit: (1) a profusion of A face particles in the median aspect of the lobe opposite the synaptic ribbon, and (2) a smooth B face devoid of particle arrangements except opposite the synaptic ridge where a single row of particles is associated preferentially with the B face of the membrane. At the lateral bulbous expansions of horizontal cell lobes, localized A face particle aggregates are present which correspond to extramembraneous densities in thin sections of this site. A structural basis for electrotonic coupling (Naka, K-I. and W.A.H. Rushton, 1967. J. Physiol. 192: 437) is afforded by a variety of arrays of gap junctions between horizontal cell processes.

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1628 SPATIAL SUMMATION IN LIGHT- AND DARK-ADAPTED GOLDFISH.
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Visual thresholds of goldfish have been measured with a range of stimulus sizes using classically conditioned inhibition of respiration. The test stimuli, circular patches of 533 nm light, were presented upon a screen 14 cms from one eye of the restrained fish and were followed by a brief electric shock to the tail. Breathing rate was assessed by a computer which also controlled the stimulus intensity in a threshold tracking procedure. Dark-adapted fish exhibited complete summation of light (Ricco's law) for stimuli smaller than a critical diameter subtending 25^{o}; with a 0.1 mL tungsten-light background, the critical diameter was 4^{o}. To show how spatial summation changes with adaptive state, thresholds to a small and a large stimulus were measured over a range of background luminances that changed the fish's sensitivity by 7 log units. Critical diameter fell from its dark-adapted value of 25^{o} to $4\text{-}6^{o}$ and remained roughly constant from 0.01 to 30 mL in spite of an apparent rod/cone break near 1 mL. This high luminance for the break point corroborates a previous finding in goldfish (1). The light-adapted critical diameter (0.32 mm on retina) corresponds in size to the smallest receptive field centers of retinal ganglion cells in goldfish (2), whereas the dark-adapted critical diameter (2 mm on retina) corresponds to the largest receptive field centers (3).

(1) Hester, F.J. (1968). Vision Res. 8, 1315-1336.
(2) Beauchamp, R.D. & Lovasik, J.V. (1973). J. Neurophysiol. 36, 925-939.
(3) Daw, N.W. (1968). J. Physiol. 197, 567-592.

1629 CONNECTIONS BETWEEN EXTRASTRIATE CORTEX AND THALAMUS IN SQUIRREL MONKEY.
Marilee Ogren* and Anita Hendrickson. Dept. Ophth., Univ. Wash. Med. Sch., Seattle, WA. 98195.

Either area 18, 19 or posterior bank of superior temporal sulcus (STS) of squirrel monkey was injected with a mixture of radioactive amino acids and horseradish peroxidase (HRP). Both reciprocal and single-direction projections could be identified in serial sections alternately processed for each technique.

Reciprocal projections were found between 18-19-STS and pulvinar. For each injection site in all three cortical areas, there was found one oval or circular ventral focus of HRP-containing neurons or silver grains and one more dorsal elongated focus. Following area 18 injections, the ventral oval focus was found in the lateral half of inferior pulvinar while the dorsal elongated focus was in the lateral half of the lateral pulvinar. Area 19 foci had the same pulvinar distribution but always lay more medially in each subdivision. The STS foci lay even more medially in pulvinar but the exact subdivisions are not yet clear.

In addition to the HRP-containing neurons which were found in the reciprocal foci, after 19 and STS injections both lateral and inferior pulvinar contained scattered large HRP-positive neurons lateral to the reciprocal focus; no silver grains were seen in this area. A second projection to area 19 and STS was shown by the presence of HRP-positive neurons in the posterior nucleus of the thalamus.

Areas 18, 19 and STS all project to the reticular nucleus of the thalamus while STS also sends terminals to deep layers of the superior colliculus.

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1630 BAR ORIENTATION DISCRIMINATION IN NORMAL AND DESTRIATED MONKEYS. Pedro Pasik, Tauba Pasik, Joanne T. Nolan* and Steve J. Solomon*. Dept. of Neurol., Mount Sinai School of Medicine, CUNY, New York, N.Y. 10029.

Four normal monkeys (Macaca mulatta) were trained to discriminate a vertical (0°) vs. a horizontal (90°) transilluminated 107 x 2 mm bar in a two-choice pulling-in situation at 380 mm distance. After achieving criterion level of performance, a threshold was determined by changing the orientation of the horizontal bar to approach the vertical, first in a tracking paradigm and then in ascending and descending series of 7 steps, 0.5° apart, around the estimated liminal level. The latter value was considered as that eliciting 75% correct responses by the method of least squares. Similar procedures were followed after histologically verified total removal of the striate cortex with partial damage to areas 18 and 19. Results: Preoperatively, the vertical vs. horizontal discrimination test was mastered with a mean of 98 errors; the mean threshold was 5.1° (SD = 3.3°). The lowest individual threshold obtained was 1.1°. Postoperatively, all monkeys reached criterion on the test with a mean of 682 errors; the mean threshold was 16.9° (SD = 9.7°). The lowest value was 8°. The postoperative increase in mean threshold was statistically significant ($p < 0.05$). When liminal values were used to estimate the number of "just noticeable differences" detected by the animals at suprathreshold levels by application of Fechner's law, it was found that the change represented approximately an 80% reduction in sensory capacity. Conclusions: Findings demonstrate a heretofore undisclosed capacity of destriated monkeys to discriminate bar orientations, and raise the question of which structures in the remaining brain are critical for the remaining ability to detect differences in orientation greater than 17° in the absence of striate cortex. Aided in part by N.I.M.H. Research Grant # MH-02261.

1631 THE OWL AND THE PUSSY CAT. John D. Pettigrew and Masakazu Konishi. Div. of Biology, California Institute of Technology, Pasadena, CA. 91125

The owl and the cat have many dissimilarities in the organization of their respective visual systems which reflect a separation of their evolutionary paths some 200 million years ago. The most notable of these differences is the absence of a partial decussation at the owl's optic chiasm.

Despite the dissimilarities, recordings from over 400 single neurons in the visual Wulst of normal and monocularly-deprived owls have established the following functional characteristics which are the same as those found in the cat visual cortex: (1) An apparent hierarchical organization with "simple" type binocularly-activated, orientationally selective cells being formed from the monocular, concentrically-organized thalamic input; (2) A high degree of binocular interaction and disparity selectivity; (3) Precise organization of both the retinotopic map and the map of preferred orientations; (4) Extreme sensitivity to visual experience in the neonatal period.

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1632 PARALLEL PROCESS FEATURE EXTRACTION. Michael Remler. Dept. Neurology, State University of New York, Stony Brook, NY 11794.

The set of inputs to a two dimensional special sensory array (a retina) is a doubly ordered set of numbers. If a feature is defined as functional on the set of all inputs with a range of zero to one, then a two dimensional array of feature detectors is also a doubly ordered set of numbers. The process of feature extraction in a sensory system then becomes the mapping of such doubly ordered arrays into themselves. These two dimensional arrays may be view as a topological vector space. A formal algebra may be define with the use of a convolution produce. The algebra may be extended with by defining a unitary operation which induces a set of linearly independent transforms. In the extended algebra a mathematical representation of a general purpose feature extraction system can be demonstrated as a polynomial in the algebra. This is neurobiologically interesting because the predicted network is a modularized parallel process structure with analogies to the known structure of the visual system. The transforms of the unitary operation may be compared to the already known special purpose detectors (eg. edge detectors). Together it provides a basis for understanding the evolution from the special purpose networks of lower animals to the general purpose network of the human brain.

1633 THE AFFERENT CONNECTIONS AND SYNAPTIC ORGANIZATION OF THE PULVINAR IN THE GREY SQUIRREL (Sciurus carolinensis). J.A. Robson* and W.C. Hall, Departments of Anatomy and Psychology, Duke University, Durham, North Carolina, 27710.

This report describes two subdivisions in the pulvinar of the grey squirrel which can be distinguished on the basis of differences in their afferent connections and synaptic organizations. The caudal part of the pulvinar receives input from large cells at the base of stratum griseum superficiale of the superior colliculus and from cells in layer VI of the temporal cortex. With the electron microscope, this subdivision is found to contain synaptic complexes consisting of a single large central dendrite which is surrounded by numerous medium sized (0.5-1.2 μm) terminals, some of which degenerate as a result of lesions in the superior colliculus. In comparison, small to medium (.3-1.1 μm) sized terminals, which contact smaller dendrites, degenerate after lesions in the temporal cortex. A rostral medial division of the pulvinar does not receive input from the superior colliculus but it does receive corticothalamic projections from areas 17, 18, and 19. These projections appear to overlap in their termination zones, but they can be distinguished, in part, with the electron microscope. The terminals of axons which originate in area 17, are large (.8-3.5 μm) and undergo neurofilamentous degeneration. They terminate in synaptic complexes resembling the complexes around the optic tract terminals in the dorsal lateral geniculate nucleus. In contrast, the axons from areas 18 and 19 have smaller terminals (.3-.9 μm) which undergo a primary dense degenerative reaction and are found outside of the large synaptic complexes. (Supported by NIH Grant NS-09623 to W.C. Hall.)

1634 EFFECTS OF BRIEF BINOCULAR EXPERIENCE ON VISUAL CORTICAL CELLS IN DARK-REARED KITTENS. P.B. Schechter and E.H. Murphy. Committee on Biopsychology, University of Chicago, Chicago, Illinois, 60637.

In normal and dark-reared 42-day-old kittens, about 80% of the visual cortical cells are binocularly responsive. This predominant binocularity may be disrupted by an imbalance of visual input to the 2 eyes: In 42-day-old kittens whose only visual experience has been 3 hours of monocular vision at 30 days of age, about 25% of the cortical cells are binocular; nearly equal numbers of cells are monocularly responsive to either eye (Schechter & Murphy, Brain Res. in press). Cortical binocularity may also be disrupted by balanced but disparate input to the 2 eyes. In the normal kitten, the eyes are divergently misaligned until the end of the 7th week of life (Sherman, Brain Res. 37, 1972). Thus, brief binocular vision during this time might disrupt binocularity.

In the present experiments, we dark-reared 4 kittens from 10 until 30 days of age. They were exposed to a bright visual environment for 3 hours and subsequently were returned to the dark until recording--at about 42 days of age. We found only 10% of 60 cells were binocularly driven; about equal numbers of cells were monocularly driven by either eye. These results demonstrate that disparate visual input--presumably with no asymmetry of ocular motility (Maffei & Fiorentini, Brain Res. 105, 1976)--is sufficient to decrease cortical binocularity. They also suggest that normal visual experience during the first postnatal weeks--prior to the onset of the "critical period"--plays an important role in the maintenance of binocularity of cortical cells during the period of ocular misalignment.

1635 BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF CHRONIC ELECTRICAL STIMULATION OF ASSOCIATION CORTEX IN CATS. Cameron J. Schlehuber, Don R. Hill* and Wm. H. Dobelle. Neuroprostheses Program, Inst. Biomed. Eng., Univ. of Utah, Salt Lake City, UT 84112.

Behavioral responses and evoked potentials were recorded from freely moving cats receiving continuous electrical stimulation to parietal cortex. Impedances remained constant for the duration of 30 days of stimulation, 12 hours per day. Stimuli (pulses 2.5 ma for 0.25 ms, plus, minus, or paired; at 50 Hz, through a 1 mu F capacitor) were delivered through 1 mm^2 platinum electrodes in a hexagonal array spaced 6 mm apart in a Teflon matrix. Initial stimulation induced petit mal seizures in about half the animals, and grand mal seizures in the remainder, with the exception of ten percent of the total which never showed signs of seizures. In 2 animals, seizures continued to occur during as well as after stimulation, these were sacrificed for histological study on their 3rd and 5th day of stimulation. The remaining animals remained seizure free for 12 hours of stimulation after an initial one or two seizures. The daily incidence of seizures declined exponentially, however, averaged evoked potentials to 10 Hz stimuli did not show any obvious changes after 30 days of stimulation.

1636 THE ANALYTIC STRUCTURE OF THE RETINOTOPIC MAPPING OF THE STRIATE CORTEX. Eric L. Schwartz* Brain Research Laboratory Dept. of Psychiatry, New York Medical College, New York N.Y. 10029.

The retinotopic mapping of the striate cortex is characterized as a (conformal) mapping of the visual field onto the cortical surface under the complex logarithm function. This summarizes in a concise way the curve of cortical magnification, the scaling of receptive field size with eccentricity, and the mapping of global visual field landmarks (meridians, octants, circles of constant eccentricity). The natural size invariance property of the complex logarithm is suggested as a possible basis for psychological size invariance in visual perception. The local functional architecture (hypercolumn structure) of area 17 is discussed in the light of the global retinotopic structure and it is suggested that the local (hypercolumn) intracortical projection of cortical afferents to simple cells is a recapitulation of the global retino-striate projection. The cortical mosaic is thus a concatenated logarithmic structure; the developmental consequences of this analysis are briefly discussed.

1637 NONLINEAR RESPONSE OF TRANSIENT RETINAL GANGLION CELLS TO RECEPTIVE FIELD CENTER ILLUMINATION INCREMENTS AND DECREMENTS. Robert P. Scobey and Leo M. Chalupa. Dept. of Behav. Biol., School of Medicine and Dept. of Psych., Univ. of Calif., Davis, 95616.

Retinal ganglion cells of the cat have been previously classified into transient and sustained types on the basis of their response patterns. Sustained and transient units are commonly thought to correspond to X and Y cells, respectively, an earlier classification which was based on linear (X) or non-linear (Y) spatial summation characteristics. Recordings from on and off-center sustained retinal ganglion cells which were stimulated by increments or decrements of a luminous spot relative to background showed a maintained change in response above and below spontaneous levels of activity for the duration of the stimulus. However, on and off-center transient cells stimulated in the same manner, showed the expected transient increase in activity, but an unexpected maintained decrease for the duration of the stimulus. For example, on-center transient cells responded with a transient discharge to spot illumination increments, but with a maintained decrease or complete suppression of activity to spot illumination decrements. It is proposed that the non-linear spatial summation manifested by the Y cells is due to a spatial summation of two neural inputs with different time functions.

1638 ANATOMY OF INTERHEMISPHERIC VISUAL CONNECTIONS IN BOSTON SIAMESE AND ORDINARY CATS. C. J. Shatz. Department of Neurobiology, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115.

The topographic distribution of neurons supplying visual fibers to the splenium of the corpus callosum was studied in Boston Siamese and ordinary cats using the method of retrograde transport of horseradish peroxidase (HRP) following localized cortical injections made through a recording micropipette. In ordinary cats, after an HRP injection at the border between cortical areas 17 and 18, which represents the vertical meridian (VM) of the visual field, HRP-labelled cells in areas 17 and 18 of the opposite hemisphere were found only immediately adjacent to the 17-18 border, thus confirming the results of previous investigations. In Boston Siamese cats the 17-18 border represents a region in the ipsilateral visual field roughly 20 degrees away from the VM, and the VM representation is displaced from the border to sites within areas 17 and 18 proper. When HRP was injected at the 17-18 border, no labelled cells were found at the border in the opposite hemisphere. Labelled cells were located instead well within area 17 near the suprasplenial sulcus, and also well within area 18. When an HRP injection was placed at the VM representation, again few HRP-labelled cells were found at the opposite 17-18 border, but instead most were found in area 17 slightly medial to the border, and in area 18 slightly lateral to it. These findings were complemented in an autoradiographic study in which orthograde transport of tritiated proline after a localized cortical injection was used to demonstrate the distribution of callosal terminals. Thus, the pattern of callosal connections revealed in Boston Siamese cats, although anatomically different from that of ordinary cats, is nevertheless consistent with the proposal that cortical sites representing similar visual field coordinates in each hemisphere are appropriately interconnected via the corpus callosum.

1639 VISUAL FIELDS OF BINOCULARLY DEPRIVED CATS FOLLOWING CORTICAL AND TECTAL LESIONS. S. Murray Sherman. Dept. Physiol., Univ. of Virginia School of Medicine, Charlottesville, VA 22901.

On a behavioral orienting test, cats raised with binocular lid suture (BD cats) have fields with each eye limited to the ipsilateral 90° (i.e., related to nasal retina), while in normally reared (NR) cats the fields extend to 45° contralateral (temporal retina)[1,2]. The BD fields are similar to those seen in NR cats made dependent on retinotectal pathways by cortical lesions[2]. Previously described techniques[1,2] were used in the present study to test 7 BD and several NR cats before and after certain brain lesions. One BD cat had an optic chiasm split to cut axons from nasal retina, and this rendered the cat blind; an NR cat with such a split oriented briskly to stimulation of temporal retina. Four other BD cats had bilateral ablations of occipitotemporal cortex (involving all known cortical visual areas), and this had a negligible effect on visual orienting. The last 2 BD cats had a unilateral occipitotemporal ablation followed later by a lesion of the superior colliculus contralateral to the ablated cortex. This resulted in stable blindness for the hemifield contralateral to the ablated colliculus with good orienting for the hemifield opposite the ablated cortex. An NR cat with such lesions oriented to stimuli throughout either hemifield. These data suggest that, for this behavior, NR cats can involve tectal and/or cortical pathways, but BD cats are largely dependent on retinotectal pathways and do not develop significant geniculocortical involvement. Since the retinotectal pathways are predominantly crossed, this could also explain the absence of clear orienting in BD cats when stimuli are presented to temporal retina.

(Supported by PHS Grants EY 01565 and EY 00020).

1. S. M. Sherman, Brain Res. *73*:491(1974).
2. S. M. Sherman, Science *185*:355(1974).

1640 RECEPTIVE FIELDS OF BINOCULAR CORTICAL UNITS AFTER VISUAL FIELD ROTATION IN DEVELOPING KITTENS. Paul G. Shinkman and Charles J. Bruce*. Univ. North Carolina, Chapel Hill, N.C. 27514.

Kittens were reared in darkness except for two hours of visual experience every day between the ages of 4 and 12 weeks. The visual experience consisted of viewing a normal environment at photopic levels of illumination through goggles fitted with small prisms, whose arrangement controlled the relative rotation between the visual fields in the left and right eyes. For one group of kittens, the visual fields were rotated 8^{o} counterclockwise in the left eye and 8^{o} clockwise in the right eye (the $+16^{o}$ condition). In a second condition, the rotations were 8^{o} clockwise in the left eye and 8^{o} counterclockwise in the right eye (the -16^{o} condition). A control group wore goggles fitted with prisms that did not rotate the visual fields (the 0^{o} condition). During the daily periods of visual exposure, informal observations were made of kittens' visually guided behavior: orienting, placing, jumping, following, and performance on the visual cliff. In general, kittens showed good visual behavior: accurate orienting toward and pursuit of visual targets, good discrimination on the visual cliff, and so forth. Subsequently, the receptive field organization of visual cortex was studied using conventional techniques of extracellular unit recording, special attention being given to the preferred orientations of binocularly activated cortical cells. For each cell, the angular difference was calculated between the preferred orientations in the two eyes; the distributions of these differences were compared among groups.

For each of the three groups, the distribution was centered about the prism rotation experienced during early development. The mean interocular disparities in receptive field orientation were $+19^{o}$ for the $+16^{o}$ condition, -21^{o} for the -16^{o} condition, and $+3^{o}$ for the 0^{o} condition; these differences are significant at $p < .001$. We conclude that the formation of binocular connections in visual cortex reflects the correspondence of early visual input to the two eyes for moderate amounts of relative rotation. Additional work now in progress suggests that goggles imposing greater values of rotation (24^{o}) disrupt binocular correspondence, as other investigators have reported using large, surgically induced eye rotations (Blakemore, 1975; Yinon, 1975).

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1641 THE ELECTRORETINOGRAM OF THE TORTOISE, TESTUDO HERMANIS. R. Siminoff, International Laboratory of Brain Research, P.O. Box 80, Kotor, Yugoslavia 81330.

The electroretinogram of the isolated retina of the tortoise, Testudo hermanis consisted of a dominant a-wave, a smaller b-wave and b-wavelets. The retina of Testudo hermanis, as with other species of turtles, is cone dominant and rods, if present, do not contribute significantly to the electroretinogram. The PIII component consisted of an initial fast (a-wave) and a later slow component. The PIII component is due, in part, to cone receptor potentials and in part to other retinal elements such as horizontal cells, bipolar cells and amacrine cells. The b-wave is probably due, at least in Testudo hermanis, to glial cells. The b-wavelet probably arises in horizontal cell-cone feedback circuits.

1642 EFFECTS OF BINOCULAR PATTERN AND LIGHT DEPRIVATION ON THE DEVELOPMENT OF EYE ALIGNMENT IN KITTENS. Diane L. Smolen
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Kittens were binocularly contour deprived and light deprived to asses the relative contribution of these stimuli on the development of interocular eye alignment in kittens. Kittens were reared in total darkness, with and without contour experience in both scotopic and photopic levels of room illumination, for three months. Pattern deprived animals wore diffusing contact lenses, while littermates wore clear lenses tinted to equate retinal illumination. Exposure periods were for four hours each day. Eye alignment was measured using a modified version of a clinical technique utilizing the corneal reflex.

Eye alignment in those animals deprived of pattern and in dark reared animals was significantly different from eye alignment measured in three month normal animals. Eye alignment in animals exposed to patterns was not reliably different from normal.

These data suggest that the development of interocular eye alignment in cats is primarily dependent upon pattern experience. The level of illumination does not seem to be important. Recovery data at three and six months will also be discussed.

1643 EFFECTS OF CORTICAL COOLING ON VISUAL AND NONVISUAL SUPERIOR COLLICULUS CELLS. Barry E. Stein and James Dixon*. Dept. of Physiology, Medical College of Virginia, Richmond, Va. 23298.

Visual, somatic and acoustic cells of the superior colliculus (SC) can receive input via ascending pathways and/or corticotectal projections. In the present experiments the influence of reversible cortical deactivation (cooling) upon the responses of these SC cells was studied in the cat. Neuronal recordings were conducted in paralyzed animals which were respired with 70% N_2O and 30% O_2. The responsiveness of the majority of superficial, intermediate and deep strata visual cells was depressed by cooling and reestablished by rewarming visual cortex. Only those visual cells with receptive fields in the central 45° of visual space were affected. Visual cells with receptive fields in far temporal space and, with few exceptions, nonvisual cells appeared to be unaffected by cortical cooling. The effectiveness of an acoustic stimulus was undiminished by cooling auditory cortex in all examples studied, and the responses of 65 of 68 cells activated by somatic stimuli were unchanged during the cooling of somatic cortex. Cooling multiple cortical regions together also failed to significantly alter responses to somatic and auditory stimuli. Thus, the simultaneous cooling of visual, somatic and auditory cortex selectively depressed visual responses in multimodal cells. The lack of corresponding effects after cooling visual, somatic and auditory cortex is surprising in view of topographical, corticotectal and functional similarities between these representations in the cat SC. These data emphasize the importance of cortical influences upon visual SC cells with central receptive fields and raise questions about the role of nonvisual corticotectal pathways.

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1644 AXON REGENERATION IN THE NEWT OPTIC NERVE. Larry J. Stensaas and Earl F. Feringa. Dept. Physiol., College of Med., Univ. of Utah, Salt Lake City, Utah and Dept. Neurol., Univ. of Michigan Med. Center, Ann Arbor, Mich.

The ultrastructural characteristics and rate of the regenerative process in the optic nerve of the newt (Triturus phyrrhogaster) was studied at extra- and intracranial sites following a freeze-crush injury near the retina. This procedure destroys cellular constituents in the extracranial portion of the nerve leaving intact the basal lamina and the blood supply to the eye. No axons were seen at the extracranial site 1-7 days post-lesion. This contrasts with the persistence of normal appearing but severed axons within intracranial portions of the nerve which thus give a false appearance of early regeneration. The first axon sprouts traverse the lesion by ten days accompanied by astrocyte processes from the retinal stump. The first regenerating axons are situated within the basal lamina and consist predominantly of axonal profiles 0.3-0.5 μm in diameter containing dense core vesicles. Axon sprouts also appear within intracranial portions of the nerve at 10 days at a time when degenerating unmyelinated axons have disappeared. The number of regenerating axons increases rapidly after 10 days with no signs of random growth at the site of injury. The small (0.2-0.4 μm in diameter) unmyelinated axons which predominate at 21 and 31 days post-lesion appear to constitute a fairly homogeneous population with few dense core vesicles and other organelles indicative of axon sprouts. These results support the view that continuity of the nerve provided by the persisting basal lamina serves to orient axon sprouts and favors an orderly process of axon regeneration without neuroma formation. Further studies are in progress to specify the ultrastructural characteristics of the axon and to determine the role of glial elements in the process of regeneration.

1645 OCULAR DOMINANCE IN LAYER IV OF THE NORMAL AND DEPRIVED CAT'S VISUAL CORTEX. M. P. Stryker and C. J. Shatz. Department of Neurobiology, Harvard Medical School, Boston, Mass. 02115.

In layer IV of the cat's visual cortex, autoradiography following transneuronal transport of tritiated proline and fucose injected into one eye has demonstrated that geniculocortical terminals from the two eyes are partially segregated (Shatz, Lindstrom, & Wiesel, Neurosci. Abstr. 1: 79, 1975). We examined the physiological correlate of this anatomical segregation by recording from single cells in identified laminae of area 17 in cats in which one eye had been previously injected with radioactive label. In layer IV of normal cats, most cells had simple receptive fields. Relatively few cells were binocularly driven, and cells were clustered according to eye preference. Transitions between the two eyes were marked with electrolytic lesions. A good correspondence was found between the distribution of cells driven from the injected eye and that of the radioactively labelled terminals.

In monocularly deprived cats, afferents representing the deprived eye are still demonstrable autoradiographically, although very few cells are reported to be driven by that eye. We wondered whether these deprived-eye terminals, while still present, might somehow be functionally suppressed. Recordings from layer IV in monocularly deprived cats also revealed a substantial input from the deprived eye; this is consistent with the anatomical findings. Furthermore, most cells driven by the deprived eye had simple or circular receptive fields. Recordings from other cortical layers showed almost total dominance by the non-deprived eye, in harmony with the results of previous workers. Almost all cells with complex receptive fields were dominated by the non-deprived eye. These findings, then, do not provide evidence that thalamic afferents representing the deprived eye are functionally suppressed in layer IV of the cat's visual cortex.

1646 SINGLE UNIT RECORDING IN THE OPTIC TRACT OF THE MEXICAN GROUND SQUIRREL: DO RODS CONTRIBUTE TO CONTRAST AND COLOR CODING? Lillian Tong* and Daniel G. Green. Vision Research Laboratory, Dept. of Ophthalmology, University of Michigan, Ann Arbor, MI 48109.

The Mexican ground squirrel has been thought to have an all cone retina. Single unit studies in the optic tract by Michael, 1968, and Gur, 1974, have revealed several types of ganglion cells and their inputs. They found that contrast and directional units had exclusive input from 525 λ_{max} cones and color opponent units had input from 460 and 525 cones. However, recent anatomical studies (West and Dowling, 1975) have shown a rod-like receptor, and ERG studies (Green and Dowling, 1975) show evidence for a 502 λ_{max} pigment. In this study, single unit recording in the optic tract of the ground squirrel shows that the 502 pigment contributes to contrast-sensitive units and to color-opponent units. Furthermore, the 502 pigment is active in dark-adapted as well as light-adapted conditions. Spectral sensitivity curves using a threshold response criterion were measured in dark-adapted, light-adapted, and chromatically adapted conditions. The most sensitive contrast units show input from 502 pigment alone. Other nonopponent units had 525 input alone, or had both 502 and 525 pigments mediating the same sign response. Color opponent units were found with a nonopponent 502 spectral sensitivity curve in the dark-adapted state and color opponency between 460 and 525 or between 502 and 525 pigments in the light-adapted state. Other units were color opponent in both dark-adapted and light-adapted conditions with opponency between the 460 and 525 pigments. Directionally selective units have not been studied. It appears that the 502 receptor has input to many types of ganglion cells. It is more sensitive than the 460 and 525 cones and is active over a wide range of background intensities. (Supported by PHS grant EY 00379 to D. G. Green.)

1647 BINOCULAR PROPERTIES OF NEURONS IN THE CAT'S POSTERO-LATERAL THALAMUS. Claude Veraart* (SPON: Michel Meulders). Lab. Neurophysiol., Univ. Louvain, UCL - 5449 B-1200 Brussels Belgium.

Binocularly driven units were recorded in the pulvinar, lateralis-posterior (LP), suprageniculatus (SG) and posterior (PN) nuclei, in "cerveau isolé" cats. Attempts were made to determine accurately visual receptive fields (VRF) related to each eye, as well as binocular interactions at various retinal disparities. An original software was developed in order to objectively point out the VRF characteristics, by means of a PDP-12 computer. With such a method, it has been possible to measure receptive field disparities, even in the case of very large VRF as described in the pulvinar, LP and SG.

About only 30% of visual cells in PN were binocularly driven, in contrast with more than 85% in pulvinar, LP and SG. Other physiological divergences were evidenced between PN and the latter nuclei: they concerned the functional organization and the proportions of visual and movement sensitive neurons. As a general rule, binocular neurons were similarly triggered without regard to the stimulated eye. Likewise, the VRF properties were similar whatever the eye considered. The values of receptive field disparities measured for single units in pulvinar, LP and SG have a range of distribution compatible with a possible role in stereopsis for these structures. Binocular interactions were investigated at various retinal disparities with different kinds of stimuli: intermittent light stimulation, moving light pattern, or VRF automatic mapping. Summations and facilitations, like an increase in the contrast between neuronal activities related to light presentation inside or outside of the VRF, were observed. Binocular properties of neurons in pulvinar, LP and SG are discussed in relation to the problem of the functional organization of these structures.

1648 EFFECT OF UNILATERAL APHAKIA ON CELL GROWTH IN THE LATERAL GENICULATE NUCLEUS OF *MACACA MULATTA*. G. K. von Noorden* and M. L. J. Crawford. Dept. Ophtha., Baylor Coll. Med. and Univ. of Texas, Grad. School of Biomed. Sc., Houston, TX, 77025

The previously reported arrest of cell growth in the lateral geniculate nucleus (LGN) following unilateral lid suture during visual immaturity in cats, dogs and monkeys could be caused by deprivation of light or form vision, binocular interaction, or a combination of these factors. The lens was surgically removed from one eye of three macaques during the first month of life. Quantitative histologic comparison between LGN laminae connected with the phakic and aphakic eye revealed reductions of cell sizes in the monocular and binocular LGN segments receiving input from the aphakic eye of a magnitude comparable to that obtained by the lid suture technique. Since the amount of light stimulating the retina through an aphakic pupil is not reduced we conclude that light deprivation can be excluded as a cause for arrest of cell growth in the LGN.

1649 UNCROSSED RETINOGENICULATE PROJECTIONS OF ALBINO VS BLACK C57BL/6J MICE. Irwin S. Westenberg*, V.A. Hosp., Phoenix, Az., U.S.A., 85012, and Donnell Creel, V.A. Hosp., Salt Lake City, Ut., U.S.A., 84113. (SPON: E. Bigler).

Albinism has been correlated with abnormal uncrossed retinogeniculate projections (RGP) in mammals. Usually RGP of an albino strain have been compared with RGP of another, pigmented strain of the same species; i.e., subjects have differed genetically not only at the albino (C) locus, but at many other loci as well. Thus the C-locus difference has been confounded with differences at other loci. To assess neuroanatomical correlates of a single-gene difference at the C locus in the absence of genetic differences at other loci, we compared albino vs pigmented mice of the same inbred strain, C57BL/6J. In this strain there have been two separate mutations to albino, c^J/c^J and c^{2J}/c^{2J}. We used 3 groups of 4 mice:
a. littermate males, 8 mos. old, 2 c^{2J}/c^{2J} vs 2 black $(+/c^{2J})$
b. littermate females, 7 wks. old, 2 c^J/c^J vs 2 black $(+/c^J)$
c. non-littermates, > 6 mos. old, 2 male c^J/c^J vs 2 female, black $(+/c^J)$.
Six or 7 days following removal of the right eyes, the mice were perfused; horizontal brain sections were stained for degenerating optic tract processes and examined on slides coded to conceal each mouse's genotype. In each group the degenerating uncrossed RGP were ranked on the basis of size, density and dorso-ventral extent in the dorsal lateral geniculate nucleus. In all 3 groups the albinos' uncrossed RGP were ranked smaller than those of their black counterparts; the probability of obtaining such rankings by chance is < 0.005 (by simple enumeration). Thus it can be concluded that in the C57BL/6J strain there are anatomical differences in RGP correlated with a genetic difference at the C locus. Since this relationship can be observed while all other genetic variables are held constant, this strain of mouse is valuable for further study of the nature of the RGP anomaly, its development and its relation to the development of other brain structures.

1650 THE INFLUENCE OF DIFFERENTIAL ILLUMINATION AND TEMPORAL LIGHT MODULATION ON BINOCULAR COMPETITION IN THE DEVELOPING KITTEN'S VISUAL SYSTEM. J. R. Wilson, S. V. Webb, and S. Murray Sherman. Dept. of Physiol., Univ. of Virginia School of Medicine, Charlottesville, VA 22901

Seven kittens were studied with electrophysiological, histological and behavioral techniques. The first 4 were reared with binocular eyelid suture. From the 3rd week to the 5th month, each had additional stimulation applied to the right eye for 1/2 to 2 hours daily. This stimulation consisted of square-wave modulated light (3 log cd/m^2 above background; modulation varied pseudo-randomly from 1-60 Hz) applied by fiber optics to the eyelids. At 7 months the kittens were studied electrophysiologically, and no interocular asymmetry was detected either in cortical ocular dominance patterns or in lateral geniculate X- and Y-cell proportions. Also, little or no differences in geniculate cell sizes were found between hemispheres for laminae A or A1. The final 3 kittens were raised with one eye covered by the lids and the second eye, by the nictitating membrane. Thus, neither retina experienced spatial patterns, but the second retina received about 1 log cd/m^2 more light than the first. At 9-18 months, 2 of these kittens had their eyes opened for behavioral tests of their visual fields. In all respects they behaved like cats raised with binocular lid suture, and no interocular asymmetry was seen. Also, geniculate cell sizes between the hemispheres for laminae A and A1 appeared equal. Therefore, in none of the 7 cats was evidence found that one eye in any way dominated the central visual pathways. We conclude that, in the absence of spatial patterns, interocular differences in the amount of illumination or temporal patterns do not provide a strong advantage to the favored eye during development of its central connections.

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1651 RELATION OF EXTRASTRIATE CORTICAL AREAS TO ATTENTION AND GAZE SHIFTS. Martha Wilson and William A. Wilson, Jr. Department of Psychology, University of Connecticut, Storrs, Ct. 06268.

The role of the extrastriate visual areas in selective attention was investigated in rhesus monkeys. Animals learned a series of color-form pattern discrimination problems with either the color or the form cues relevant. After each problem was mastered, correct response required responding to the previously irrelevant dimension. On half the problems, the two stimulus dimensions occupied the same spatial locus while on the other half, the two dimensions occupied different spatial locations. Matched groups of three monkeys received lesions of inferotemporal (IT) or prestriate (PS) cortex, or cortex in the bank of the superior temporal sulcus (STS) described by Zeki (1969). A fourth group served as normal controls (NC). The groups differed overall in their ability to perform the shift tasks ($p=.02$). Further analysis of performance showed that different processes were disturbed in the various lesion groups. The IT group took more trials to extinguish previously rewarded responses ($p=.02$). In acquisition of a new attentional response, the IT and NC group performed better when the shift in response involved an inferred shift in gaze, while the STS group performed better when the relevant cues occupied the same position in space as the previously rewarded cues. The PS group showed no difference on the two types of shift problem. The interaction between lesion groupand type of shift was significant ($p=.02$)

These results provide further evidence for different functional roles of IT and PS cortical areas. In addition, they implicate an area in the superior temporal sulcus as important for shifts of attention in space.

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