NEUROSCIENCE
Abstracts

VOLUME II

Part 1

NEUROSCIENCE ABSTRACTS

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ABSTRACTS

Audition

1 DIFFERENTIAL PROJECTIONS OF ASCENDING INPUTS TO THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS. L. M. Aitkin,* L. Roth,* and M.M. Merzenich* (SPON: H. J. Ralston III). University of California, San Francisco, Ca. 94143

The central nucleus of the cat inferior colliculus has a tonotopic organization and a relatively simple, laminated architecture, yet it receives ascending input from at least ten lower auditory structures. In order to determine if these auditory nuclei project differentially upon the central nucleus, within the framework of a strict cochleotopic pattern, a series of parallel physiological and anatomical studies were conducted in the central nucleus. Microelectrode mapping techniques were employed to determine the effectiveness of ipsilateral, contralateral, and binaural stimulation at different central nucleus loci. In anatomical studies, horseradish peroxidase was injected at physiologically-defined sites to ascertain which lower structures project to those loci.

Microelectrode experiments confirmed the predominantly binaural innervation of the central nucleus but also revealed that discrete regions within the nucleus received a purely contralateral input. Horseradish peroxidase injections commonly led to uptake of the tracer by most of the lower auditory nuclei known to project to the central nucleus. Reconstruction of these results indicated that neurons projecting to the central nucleus were topographically related to the location of the injection site. Furthermore, for injections in which a projection from the superior olivary complex was revealed, labelled cells were not only observed at the appropriate topographic, i.e. tonotopic, region in the olivary nucleus, but were also distributed throughout the entire rostrocaudal extent of these nuclei.

The number of labelled neurons in a given projecting auditory nucleus varied markedly from experiment to experiment. For example, in some cases no neurons were labelled in the contralateral cochlear nucleus, while in other cases, labelling of neurons in the cochlear nucleus was massive. Similiarly, in some injections few or no neurons were labelled in the superior olivary complex, while in others, superior olivary labelling was prominent. Of the projecting nuclei, the input from the ipsilateral nuclei of the lateral leminiscus was the most constant, i.e., it invariably provided a significant proportion of labelled neurons.

These experiments suggest that a given discrete region of the central nucleus might receive a dominant input from either the contralateral cochlear nuclei or the superior olivary complex, indicating that some form of differential projection from these structures exists. The significance of these findings, which are shown in the context of the highly ordered tonotopic organization of the central nucleus, will be discussed.

Supported by NIH grant NS10414

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2 EFFECT OF AUDITORY NEOCORTEX ABLATION ON BINAURAL MASKING-LEVEL DIFFERENCES IN THE CAT. J. L. Cranford, J. Stramler, * M. Igarashi, Dept. Otorhinolaryngology & Communicative Sciences, Baylor College of Medicine, Houston, Texas, 77025.

The "binaural masking-level difference (BMLD)" phenomenon in binaural hearing refers to the observation, obtained in a large number of experiments with human subjects (see Green & Henning, 1969, for a recent review), that the detectability of an auditory signal presented in noise may be improved by appropriate changes in the interaural relations between either the signal or the noise. Two recent experiments by Wakeford & Robinson (1974) and Cranford (1975) demonstrated the occurrence of BMLDs in normal cats. Cranford measured the effects of noise at one ear on the detection thresholds for tones embedded in noise at the opposite ear, whereas Wakeford & Robinson measured the relative detectability of in-phase (diotic) vs. 180^{o} out-of-phase (dichotic) binaural tones in the presence of continuous diotic noise. Cranford found that cats exhibit significantly lower detection thresholds for monaural tones when noise is present in the nonsignal as well as the signal ear; Wakeford & Robinson, on the other hand, observed improved detectability of tones when they are out-of-phase relative to the noise. The purpose of the present experiment was to examine the effects of unilateral and bilateral ablation of auditory cortex on these two forms of BMLD test.

Seven cats, wearing stereo headsets, received each of the two BMLD tests prior to, and after each stage of a two-stage ablation of auditory subdivisions AI, AII, Ep, SII, and I-T. Two additional cats were tested before and after one-stage bilateral operations. Preoperatively, the mean BMLD with the Cranford test was 4.9 dB (range of 2.9 to 6.7 dB for individual cats); in the Wakeford & Robinson test the mean was 6.7 dB (range of 4.4 to 8.4 dB). After unilateral operations no significant changes were observed with either of the two BMLD tests. After bilateral lesions, however, it appears that, while BMLDs are still present, they are reduced in magnitude to levels below that observed prior to the first operation. With the Cranford test the mean BMLD was reduced to 2.6 dB (range of 2.1 to 4.5 dB); with the Wakeford & Robinson test the final mean BMLD was 4.6 dB (range of 2.8 to 6.7 dB). Thus, it appears that binaural masking-level differences, like sound localization (Ravizza & Masterton, 1972; Heffner & Masterton, 1975), can be observed in animals deprived of auditory neocortex.

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Supported by NINCDS Grants NS11812 and NS10940.

3 A MEASURE OF CENTRAL AUDITORY PROCESSING IN HUMANS. DD Daly, DM Daly, RJ Roser*, J Godfrey* and K Millay*, Callier Ctr. Comm. Dis., U.T. Dallas, and Dept. Neurology, UT Health Sci. Ctr., Dallas; Dallas, Texas 75235.

Several lines of evidence suggest the existence of neuronal systems selectively sensitive to complex auditory stimuli having acoustic features similar to speech sounds. Such systems may provide the basis for acoustic processing which is exploited in speech perception. If so, disorders of such processing should be reflected in impaired speech perception, as well as in other behaviors.

In normal subjects studies of identification and discrimination of synthetic sounds resembling English consonants indicate such rapidly changing stimuli tend to be perceived "categorically". Using sets of stimuli like those of Liberman et al. (J. Exp. Psychol. 52:127, 1956) we replicated the identification functions (IF) which they found in normals and have extended the study to other populations. We use two-choice forced response identification tests with 2 sets of 12 stimuli in which the time for frequency change to steady state varies in 10 msec steps from 10 to 120 msec. Depending on the second formant frequency, one set was perceived as |bɛ|-|wɛ| and the other as |gɛ|-|yɛ|. Stimuli, recorded on tape in randomized sequences, were presented monaurally to each ear and binaurally. Normal subjects tend to identify the shorter stimuli as the same (e.g.|bɛ|) and the longer stimuli as the same (e.g. |wɛ|), with an abrupt narrow range of uncertainty (e.g. at about 40 msec).

With these sets of fixed stimuli we have explored the range and stability of perception in an abstract auditory space (domain) in certain populations of patients and subjects, all of whom had essentially normal hearing sensitivity by pure tone and speech audiometry. We have found alterations from normal IF in the following populations:

1) 9 adults (19-63 years) with acquired cerebral lesions (7 left hemisphere; 2 right). Altered IF were present in three patients without aphasic defects in language; two patients with right hemisphere lesions, and one patient with a left hemisphere lesion who had only severe auditory comprehension problems ("word deafness"). One patient with severe anomia but no comprehension problems had normal IF. The remaining five patients had both altered IF and aphasia. All patients in this group had reported normal hearing before the onset of their illness.

2) 2 adults of superior intelligence who could not learn to articulate foreign languages or to recognize their own speaking errors in these languages, despite a knowledge of the languages sufficient for reading and writing. Both had normal speech discrimination scores. One subject showed normal IF for |bɛ|-|wɛ| but "mirror" or inverted identifications for |gɛ|-|yɛ|, the sounds she could not articulate properly in German or French. The other subject was incapable of discriminating |bɛ| and |wɛ| even at the extremes of the set. The |gɛ|-|yɛ| set was identifiable but not "biquantal".

3) 8 children diagnosed as having "auditory dyslexia": 3 siblings, a father-son kinship, and four unrelated children. All children had altered IF as did the father in the kinship. In some of the children, only one of the stimuli sets was misidentified.

These studies provide evidence of: (1) Central auditory processing (CAP) at cortical levels which may be disrupted by discrete acquired lesions. (2) Defects in processing at prephonetic and prelinguistic levels since altered IF occurred in subjects with normal speech discrimination scores and without aphasia. (3) Differences in CAP in normal subjects and, thus, evidence of different auditory perceptural domains, resulting in differences in representation of phonetic (speech) information.

We believe this method constitutes a sensitive clinical test of CAP. Rigorous control over acoustical parameters of the stimuli permits development of a heirarchy of such tests.

4 CENTRAL AUDITORY PROCESSING IN PATIENTS WITH ACQUIRED LESIONS OF CEREBRAL HEMISPHERES. DM Daly, DD Daly, RJ Roser*, J Godfrey* and K Millay*, Callier Ctr. Comm. Dis., UT Dallas and Dept. Neurology, UT Health Sci. Ctr. Dallas, Texas 75235.

Disruptions of neural systems selectively sensitive to complex auditory stimuli with acoustic characteristics similar to speech may occur in certain communication disorders. Using synthesized acoustic stimuli as described in the preceding abstract, we have studied alterations of identification functions (IF) in patients with acquired lesions in the cerebral hemispheres.

We have examined 9 adults, 19 to 63 years of age, with acquired focal lesions, seven in the left cerebral hemisphere, two in the right. All patients reported normal hearing before the occurrence of their lesions. On examination all had hearing sensitivity essentially within normal limits as determined by pure tone and speech audiometry; seven had speech discrimination scores within normal limits. We tested each patient on several occasions, some over intervals of several months, with the last study at a time when the lesion was static. Of the patients with left hemisphere lesions, five had suffered cerebral infarctions, one had had a brain abscess, and one had had traumatic contusion. Of the patients with right hemisphere lesions, one had undergone temporal lobectomy for intractable seizures due to a low grade glioma, the other had had a brain abscess.

Alterations of IF may occur independently of aphasic defects. Both patients with right hemisphere lesions showed altered IF. One patient with a left hemisphere embolic infarction and severe defects in auditory comprehension, but without aphasic defects either in oral language production or in comprehension and production of written materials ("word deafness"), showed severe alterations of IF. One patient with a left hemisphere infarction and severe anomia but no comprehension defects, showed normal IF. The remaining patients exhibited both altered IF and aphasia.

Altered IF often included loss of the "biquantal" pattern seen in normals. Occasionally, a "mirror" IF was observed. This did not appear to be mislabeling due to aphasia since it was observed for only one set of stimuli and remained stable over testing sessions. One patient with a right hemisphere lesion was unable to discriminate between |bɛ| and |wɛ| even at the extremes of the set, reporting all stimuli from that set as |bɛ|.

Alteration of IF occurred usually, but not solely, in the ear contralateral to the lesion. Several patients showed persistent differences between ears with monaural stimulation. The patient with "word deafness" showed markedly altered IF for |gɛ|-|yɛ| only with the ear (R) opposite the lesion; she had discovered that she could comprehend telephone conversations better when using the left ear. Despite intact IF, the patient with anomia showed a persistent shift of the IF boundaries, in the direction of the longer stimuli, with the right ear. In several patients, binaural IF differed from both monaural IF and could not be described by arithmetic summation of simple monaural IF. However, these may be amenable to analysis as power functions.

These studies provide conclusive evidence of altered central auditory processing due to single, acquired focal lesions in the cerebral hemispheres. Such altered processing occurs independently of aphasic defects and is prephonetic and prelinguistic. These findings can provide an independent basis for design and evaluation of aphasia therapy. The tests appear highly sensitive, yet can be administered in the presence of severe aphasic defects. A heirarchy of such tests may refine lateralization and localization of lesions.

5 EFFECTS OF PROBENECID ON NOREPINEPHRINE IN CSF. Charles R. Lake, Michael G. Ziegler*, James H. Wood*, Michael H. Ebert*, and Irwin J. Kopin. Laboratory of Clinical Science, NIMH, Bethesda, Md. 20014.

Probenecid competitively inhibits active transport of acidic metabolites of monamines from the cerebrospinal fluid (CSF) to blood. Eighteen hours after probenecid administration, CSF levels of 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), the catabolites of serotonin (5-HT) and dopamine (DA), are increased by 450% and 875%, respectively. The accumulation rates of these metabolites are used as indices of the rates of utilization of the parent amines, and have been studied in various neuropsychiatric disorders. CSF levels of MHPG (4-hydroxy-3-methoxyphenylethyleneglycol), the major catabolic product of norepinephrine (NE) in brain, are increased by 60% after probenecid administration. MHPG, however, is a neutral compound not removed by the acid transport system, and its increase after probenecid is unexplained.

In this study the effects of probenecid on CSF and plasma levels of NE are examined. Blood (12 ml) and lumbar spinal fluid (4 ml) were obtained from 20 neurological patients before and eighteen hours after probenecid. Plasma and CSF levels of NE were measured by a radioenzymatic assay and CSF levels of probenecid were determined by gas chromatography.

Probenecid administration resulted in an increase of plasma NE from 392 $\pm$ 35 pg/ml ($\pm$ SEM) to 504 $\pm$ 57 pg/ml with a mean increment of 112 $\pm$ 37 ($p < 0.005$, matched paired t test). Levels of NE in CSF increased from 270 $\pm$ 37 to 375 $\pm$ 45 pg/ml after probenecid, with a mean increase of 105 $\pm$ 19 ($p < 0.0005$). The CSF probenecid level was 17.53 $\pm$ 1.54 µg/ml. There was no significant relationship between levels of probenecid and increments in plasma or CSF levels of NE. NE levels in CSF, however, were significantly related to those in plasma (LR = 0.535; $p < 0.05$) both before and after administration of probenecid. The increases in NE levels in plasma were also correlated significantly with those in CSF (LR = 0.611; $p < 0.01$), although plasma NE does not normally enter CSF.

The increment in levels of amine metabolites in CSF after probenecid administration is a valid indication of the normal rate of amine utilization only if the drug blocks acid transport and does not influence the rate of amine utilization. Probenecid is not known to block transport or metabolism of amines so that the increment in levels of NE in CSF and plasma must be attributed to an increase in release of the amine. Since there does not appear to be a relationship between levels of probenecid in CSF with the increments in NE in plasma or CSF, it is unlikely that the drug directly effects amine release. The results suggest that stress, associated with the varying discomforts of probenecid administration, is responsible for elevation of the NE levels and may account for the increased CSF levels of the NE metabolite, MHPG, after probenecid administration. These observations also raise questions about the contribution of stress attending discomforts associated with drug administration to the increments in metabolites of DA and 5-HT found after probenecid administration.

6 MIDBRAIN PROJECTIONS TO THE MEDIAL GENICULATE BODY AND THEIR RELATIONSHIP TO CORTICOFUGAL PROJECTIONS OF THE AUDITORY CORTEX. D.L. Oliver, Departments of Anatomy and Psychology, Duke University, Durham, N. C. 27710.

The medial geniculate body (MGB) of the tree shrew (Tupaia glis) contains at least seven cytoarchitectonically distinct subdivisions, each of which is connected reciprocally to an equally well-defined area within the auditory cortex (Oliver, D.L. and Nelson, R.J., Neurosci. Abs. 1:34, 1975). Previous experiments demonstrated that these subdivisions can be distinguished on the basis of their afferent connections from the inferior colliculus and other midbrain structures (Oliver, D.L. and Hall, W.C., Brain Res. 86:217-227, 1975). The present report will present the results of a more refined analysis of the locations of the midbrain cells which project to the MGB and will relate these projections to the corticotectal and corticotegmental connections of the auditory cortex. Both anterograde and retrograde axonal transport methods (autoradiography and horseradish peroxidase) as well as anterograde degeneration methods were used.

The projections to the medial geniculate from the inferior colliculus (IC) are best understood when the colliculus is described as a set of cytoarchitectonically distinct nuclei. It is then seen that each nucleus is specifically related to a subdivision of the MGB. For example, the central nucleus of IC projects to the ventral nucleus of MGB. Both nuclei possess laminae composed of cells with disc-shaped dendritic fields, and the connections of the two nuclei suggest a point-to-point topography between their laminae. Likewise, the nuclei of the "rind" surrounding the central nucleus of IC project to specific targets in MGB. The roof of the colliculus projects to the deep dorsal nucleus of MGB, while the medial nucleus of the colliculus projects to the caudal-marginal nucleus of MGB. The lateral zone of the colliculus projects to the rostral part of the medial division of MGB. Unlike the other subdivisions of MGB, the caudal part of the medial division receives inputs from all of the collicular subdivisions. The medial division also receives inputs from the contralateral central nucleus and parts of the midbrain tegmentum. Cells projecting to the suprageniculate nucleus of MGB are located outside of the inferior colliculus in the intermediate and deep layers of the superior colliculus, in the tegmentum medial to the brachium of the IC, and in the cuneiform nucleus beneath the IC. Cells which project to the dorsal nucleus of MGB originate chiefly in the sagulum and in the midbrain tegmentum lateral to the tectopontine fibers.

The cortical projections to these midbrain areas can also be related to the subdivisions of the medial geniculate body. In some cases, a cortical area projects back to the same midbrain region which, in turn, projects to that cortical area via its relay in the MGB. For example, the cortical target of the suprageniculate nucleus projects back to the deep layers of the superior colliculus but not to the inferior colliculus. Likewise, the cortical target of the dorsal nucleus of MGB projects to the "rind" of the inferior colliculus, but its heaviest projections are to the lateral midbrain tegmentum and sagulum. On the other hand, the corticofugal projections of the primary cortex are an exception to this pattern. The primary cortex, the target of the ventral nucleus of MGB, does not project to the central nucleus of the inferior colliculus but rather to the "rind" which surrounds it. The primary cortex terminals are deeper in the "rind" than those of the cortical target of the dorsal nucleus. The functional role that the corticofugal projections may play is unclear at this time; however, it may be that a cortical area projects to that midbrain region from which it can best influence its eventual ascending inputs via the thalamus. (Supported by NIH Grant NS-09623 to W.C. Hall.)

7 BRAINSTEM AUDITORY EVOKED RESPONSES (BAER'S) IN THE RAT--EFFECT OF FIELD EXTENSION, ASPHYXIA AND AUTOIMMUNE ENCEPHALOMYELITIS. Victor F. Schorn*, Vanda A. Lennon and Reginald G. Bickford. Dept. Neurosciences, UC San Diego, CA. 92037 & Salk Institute for Biological Studies, La Jolla, CA. 92037.

The far-field evoked responses in the rat, (first observed by Jewett and Romano, (Br.Res. 36,101,115, 1972) have been examined using standard recording and averaging techniques under four conditions:

1. Wave components in the normal animal: In normal rats, four to seven reproducible waves have been observed with the following latencies: Wave I 1.3 msec s.d. .1 msec, Wave II 2.2 msec s.d. .1 msec, Wave III 2.9 msec s.d. .1 msec, Wave IV 4.1 msec s.d. .2 msec, Wave V 5.0 msec s.d. .2 msec, Wave VI 6.1 msec s.d. .2 msec (Figure below).
2. Field extension experiments: After surgical removal of the cranium, the evoked potential field was allowed to extend into a large (4x4 cm) saline pool and the evoked potential field was mapped directionally using an exploring dipole and stereotatic control. The different wave components can be shown to have individualized vector fields within the saline pool and the slope of the potential field across the brain-saline interface has been examined.
3. Suppressive effect of asphyxia on BAER and EEG: Suppression of BAER's occurs after that of the EEG (high gain) under conditions of asphyxia. The early waves of BAER increase in latency after EEG suppression. The later waves of the BAER are differentially sensitive to asphyxia suppression.
4. Experimental autoimmune encephalomyelitis (EAE): A hyperacute form of EAE (HEAE), a model of acute necrotizing leukoencephalopathy in man, is induced in Lewis rats by immunization with guinea pig myelin basic protein with Freund's complete adjuvant and B. pertussis vaccine (Fed.Proc 34:950,1975). Rats recorded in the active phase showed: (a) prolonged latency shifts, in some cases unilateral, (b) changes in the amplitude of components. The evoked potential pattern was normal in control rats injected only with adjuvants and in rats with the milder ordinary form of EAE (analogous to acute disseminated encephalomyelitis) which is induced when B. pertussis is omitted.

(Supported by USPHS NS 08962 and the National Multiple Sclerosis Society)

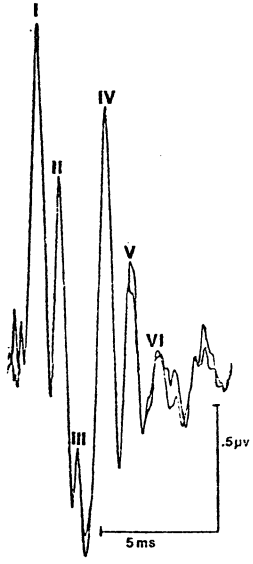


Figure 1.

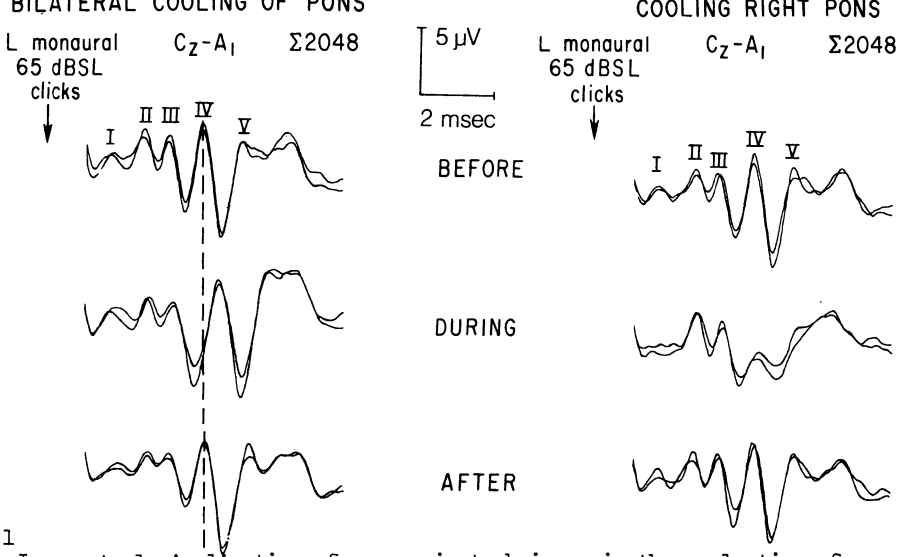
BAER of a normal rat anesthetized with pentobarbital. Recorded vertex to right ear. Clicks delivered to the right ear at 20 per sec. and at 60 dB. 2000 responses averaged.

8 EFFECTS OF FOCAL BRAINSTEM COOLING ON THE FAR-FIELD ACOUSTIC RESPONSE IN THE CAT: RELEVANCE TO RESPONSE ABNORMALITIES IN CNS DEMYELINATING DISEASE
J.J. Stockard, V.S. Rossiter and T.A. Jones. Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla CA 92093

Two patients with evidence for single, large, bilaterally symmetric foci of demyelination in the pons showed marked prolongation of all components of the far-field acoustic response after wave III; this delay relative to earlier waves tended to be constant for each of the later waves. Eight other patients had evidence for multifocal brainstem demyelination: five of these patients had prolonged latency of wave IV-V (these two waves usually fuse in man) and, in four of these, other waves were also delayed from their expected latencies -- the delay increasing progressively for successive waves; three of the patients with multifocal demyelination had reduced amplitude of wave IV-V without prolongation in latency of this component.

In the cat, the constant latency shift of all waves after III could be reproduced by cooling the pontine auditory pathway below the nuclei of the lateral lemnisci to 8°-15° C., a technique described elsewhere [1]. Simultaneous bilateral cooling of this and more rostral levels of the brainstem auditory pathway produced progressive delays in the latencies of waves after III without reducing response amplitude (Figure, left). Unilateral cooling of the auditory pathway just caudal to the nuclei of the lateral lemniscus -- and contralateral to monaural click stimulation -- greatly reduced wave IV amplitude but only slightly prolonged latency (Figure, rt.).

These findings suggest that the relative degrees of latency or amplitude abnormality in the far-field acoustic response of patients with brainstem demyelination may relate to the symmetry of pathway involvement at a given brainstem level and to the number of levels involved. Bilateral cooling or demyelination would tend to symmetrically slow conduction in ascending auditory tracts on both sides of the brainstem, delaying the activation of more rostral response generators but maintaining the bilateral synchrony of activation (and amplitude of resultant far-field potentials). On the other hand, unilateral slowing would alter phase relationships on the two sides and desynchronize response generators above the level of the lesion, affecting the amplitude relatively more than the latency of corresponding far-field potentials. Slowing between more than one pair of such generators (multi-level lesions) could produce the progressive latency shifts.



1 Jones et al: Application of cryogenic techniques in the evaluation of coma mechanisms and afferent pathways. Proc 1976 San Diego Biomed Symp, in press.

9 FUNCTIONAL ENIGMA OF COCHLEAR OUTER HAIR CELLS. J. J. Zwislocki and W. G. Sokolich*. Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

One of the striking features of the mammalian auditory system is its sensitivity to changes in sound frequency. The sensitivity is attributed primarily to a mechanical sound filtering in the cochlea that is sharpened at the level of first-order neurons. The neural sharpening mechanism has not been elucidated thus far. Of the two populations of cochlear receptors, the outer hair cells outnumber the inner hair cells in a ratio of about 3 to 1. In spite of their numerical minority, the inner hair cells are innervated by nearly 95% of the afferent neurons of the auditory nerve. Consequently, practically all single-unit recordings from the nerve must be assigned to these neurons. Because it is not certain that neurons innervating the outer hair cells have ever been recorded from, the functional role of the outer hair cells has remained enigmatic. Nevertheless, evidence has been accumulating in favor of an indirect effect of the outer hair cells on the activity of the fibers innervating the inner hair cells. For instance, the frequency selectivity (tuning) of afferent fibers is reduced considerably when outer hair cells are selectively eliminated through the use of ototoxic drugs. We performed a series of experiments to investigate the suspected interaction between the inner and outer hair cells. The experiments concerned primarily responses of individual auditory-nerve fibers to low-frequency trapezoidal deflections of the basilar membrane. Thus far, the recordings obtained from Mongolian gerbils, our preferred experimental animals, have lent themselves to only one explanation, namely, that the inner and outer hair cells do interact, and that the contribution of the outer hair cells involves an inhibitory synapse. This outcome has led to a model which accounts for the neural sharpening of the mechanical cochlear frequency analysis and for other hitherto unexplained response characteristics of the cochlear nerve. However, a clear anatomical substrate for the suspected interaction between the inner and outer hair cells remains to be found.

10 FIELD ANALYSIS OF AUDITORY BRAINSTEM RESPONSES. L. Joseph Achor* (SPON: A. Starr). Department of Psychobiology,UCI, Irvine, CA. 92717.

The present study was designed to evaluate the hypothesis that some of the waves of scalp-recorded far field acoustic responses might represent "multiple generators in algebraic summation." Clicks were presented monaurally at 25/sec to anesthetized cats held in a stereotaxic frame. Evoked responses were recorded in the configurations 1) vertex referenced to neck, and 2) depth macroelectrode referenced to neck. Isopotential maps were constructed for each of nine sagittal planes at times corresponding to peaks in the scalp recording to locate the contributing neural generators.

Preliminary findings indicate that Waves II,IV, and V are generated by activity occurring in two or more regions of the brain, whereas Wave III is generated by only one region of the brain. Wave I is not detectable using the vertex to neck recording configuration. Wave II, which consists of a positive peak and a positive inflection on the rising phase is generated by the ipsilateral cochlear nucleus and the contralateral superior olivary complex. Wave III has only one significant contributor located contralaterally, but it has not been confirmed whether the generator is the superior olivary complex or the lateral lemniscus. The contralateral superior olivary complex and the bilateral inferior colliculus are the main contributors to Wave IV. The trough of Wave IV and the peak of Wave V both receive a contribution from the lateral lemniscus, bilaterally. Wave V receives an additional contribution from the inferior colliculus, bilaterally. These findings support the hypothesis of multiple generators for several of the components of the far field acoustic response and suggest that the concept of single principal contributors for each of the waves is an oversimplification.

11 CENTRAL PROJECTIONS TO THE COCHLEAR NUCLEUS. Joe C. Adams. LNO, NINCDS, NIH, Bethesda, MD. 20014.

Neurons which project to the cochlear nucleus of the cat were identified after injecting horseradish peroxidase in the nucleus. Small cells interconnect the anteroventral and dorsal cochlear nuclei. Crossed connections originate in the contralateral anteroventral, posteroventral, and deep parts of the dorsal cochlear nuclei. Descending projections originate bilaterally in periolivary nuclei, nuclei of the lateral lemniscus, and the inferior colliculi. Periolivary cells which project to the cochlear nucleus form a continuous network around and within the principal olivary nuclei and extend from the posterior portion of the ventral nucleus of the lateral lemniscus to the inferior olive. The locations of periolivary cells projecting to a given side are roughly symmetrical about the midline. Most are around the lateral superior olive and in the ventral nucleus of the trapezoid body. Projections arise from the central nucleus and from extracentral portions of the inferior colliculi and have as their principal target the dorsal cochlear nucleus.

12 FREQUENCY RESPONSE CHARACTERISTICS OF ELECTRORECEPTORS IN "PULSE TYPE" ELECTRIC FISH. Joseph Bastian. Dept. Zoology, Univ. Okla., Norman, OK. 73069

One category of weakly electric fish produces an electric organ discharge (EOD) consisting of brief pulses separated by longer intervals. Electroreceptors in these fish were found to be most sensitive to stimuli having frequencies similar to the dominant frequency components of the species specific EOD. The population of electroreceptors could be divided into two major categories. One category, pulse marker receptors, responded to suprathreshold stimuli with one spike at a short latency (< 2 msec). The second category, burst-duration coders, responded with a burst of spikes at a longer latency. The first category of receptors was sharply tuned to the higher frequency components of the EOD pulse of a given species and were always 5 to 10 dB less sensitive than any other receptor categories. The second category, burst-duration coders, could be further divided into 3 sub-categories. In one type, rarely seen, the tuning was similar to that in pulse marker neurons. The second sub-category was more broadly tuned and the best frequency coincided very well with the major frequency component of the species specific EOD. The third category was most sensitive to lower frequency components that were weakly if at all represented in the species EOD. It is suggested that the diversity in the frequency response characteristics of various receptor types allows the animal's to identify and evaluate signals resulting from their own EOD and the EOD of conspecifics as well as electrical stimuli generated by other species of electric fish. Supported by NIH Grant # 1 R01 NS 12337.

13 SINGLE UNIT RESPONSES IN THE AUDITORY CORTEX OF MONKEYS SELECTIVELY ATTENDING STIMULI TO ONE EAR. Dennis A. Benson* and Robert D. Hienz* (SPON: M. H. Goldstein, Jr.). Dept. of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, Md. 21205.

This study examined whether neurons in the auditory cortex are affected by selectively directing an animal's attention to stimuli occurring in one ear. Acoustic stimuli consisting of tones, noise, or click trains 100 msec in duration were presented through headphones randomly to the left or right ear of rhesus monkeys. Each ear was alternately selected as the "attended" ear in blocks of 100 trials by illuminating either a left or right response key. For example, when the right ear was to be attended the animal was trained to press the right key once for each stimulus in the right ear (attend) and not to respond to left ear stimuli (non-attend). Comparisons of the activity evoked by stimuli to a given ear under attend and non-attend conditions were made for 77 units in the auditory cortex (koniocortex and adjacent secondary auditory fields) of three rhesus monkeys. For the population of units the rate of evoked discharge significantly increased for the attended stimuli, even though 36% of the units decreased their evoked activity for the attended stimuli. The changes in evoked activity were not accompanied by changes in the basic temporal response pattern or by changes in spontaneous activity. For some units, the changes in evoked discharge rate between the attended and non-attended conditions occurred as early as 30 msec after the onset of the auditory stimulus. (Supported by NSF Grant No. BMS74-19040)

14 EFFECT OF CHOLINERGIC DRUGS ON THE AUDITORY EVOKED RESPONSES FROM BRAIN STEM TO AUDITORY CORTEX. V.K.Bhargava*,A.Salamy,A.Amochaev*and C.M.McKean. Brain-Behavior Research Center, LPNI (UCSF), Eldridge, CA 95431 (U.S.A.).

Numerous investigations have been concerned in demonstrating the role of chemical substances especially acetylcholine in central synaptic transmission. However, very few studies have attempted to relate the electrophysiological events in the auditory pathway (from the brain stem relays to the cortical receptors and association pathways) to cholinergic manipulations. The present studies were therefore undertaken to study the effect of drugs modifying cholinergic transmission on the brain stem nuclei (far-field) and cortical components of the auditory evoked responses, with a view to evaluate the cholinergic mechanisms involved in modulation of a sensory impulse in the auditory pathway. The auditory evoked responses were recorded from rats with durally-implanted electrodes. The typical evoked potential from the surface of the auditory cortex consisted of two positive and two negative waves (P1, N1, P2 and N2) occurring at 9.8+4.1, 26.9+5.3, 73.1+10.6 and 135.2+21.3, latencies respectively. Eserine (.05-.2 mg/Kg) increased the amplitudes of all waves. The effect was more marked on N1, P2 and N2. It also decreased the latencies of P2 and N2. Similar effects on the amplitudes of N1, P2 and N2 were seen with carbachol and oxotremorine. Atropine (2.5-10mg/Kg) increased latencies of P2 and N2 and decreased amplitudes of N1, P2 and N2. The far-field responses of the brain stem consisted of 4-6 positive waves (.8-7msec). Eserine in low concentration (.05-.1mg/Kg) caused a decrease in the latency and amplitude of 3 and 4 waves, a high concentration (.2-.6mg/Kg) increased latency and amplitude of 2, 3 and 4 waves. This biphasic effect was more marked with carbachol (.1-5mg/Kg). Atropine did not affect latencies but decreased the amplitudes of far-field potentials. The present results thus provide evidence of involvement of cholinergic mechanisms in modulation of far-field and cortical auditory evoked responses. (Supported be NIH #HD01823.)

15 HRP UPTAKE IN GUINEA PIG ORGAN OF CORTI. William E. Brownell, James W. Fleshman Jr., Marc S. Karlan. Dept.'s of Neuroscience and Surgery (ENT), Univ. of Florida, Gainesville, Fla., 32610.

Horseradish peroxidase (HRP) dissolved in a modified Ringer's solution was perfused into the perilymph of guinea pig cochleas. Animals were placed in a sound-insulated room and either exposed to relatively high intensity broad-band noise or maintained in silence. Cochlear microphonics were monitored and showed changes of less than 10 dB for up to two hours post-perfusion as compared to pre-perfusion values. The cochlea was dissected and surface preparations of the organ of Corti were examined under phase contrast and darkfield illumination. HRP reaction product could not be found in the inner hair cells of those animals maintained in silence but was present in the inner hair cells of sound exposed animals. While reaction product was routinely found in outer hair cells, its uptake pattern varied with sound stimulus conditions. The observed HRP uptake in outer hair cells resembles that of retinal photoreceptors as described by S.M. Schacher, et. al. (Nature, 249:261, 1974). The results imply that outer hair cells are synaptically active in the absence of an acoustic stimulus. Stimulus related change of synaptic activity may reflect the modulation of a "silent current" across the organ of Corti. Displacement of the basilar membrane may increase the impedance across the outer hair cells and shunt the current to inner hair cells causing increased neurotransmitter release at the inner hair cell- eighth nerve synapse. This suggests that the relation between outer hair cell synaptic activity and eighth nerve discharge is reciprocal. (Supported by USPHS grant 1 R01 NS 12209-01 to W.E. Brownell and USPHS training grant 5T01-NS 05385-14).

16 THE NEUROBIOLOGY OF THE ACOUSTIC TUBERCLE IN TUPINAMBIS NIGROPUNTATUS. R. Browner and D. Caspary. N.Y. Medical College, Valhalla, N.Y. and S.I.U. Medical School, Springfield, Illinois.

The cytoarchitecture of the acoustic tubercle (AT) was analyzed in 14 animals. Brains were stained with cresyl-violet. Golgi Kopsch impregnations were embedded in Araldite and sectioned in the standard planes between 40-160μm.

The AT is located in the dorsomedial portion of the medulla. Nucleus laminaris (NL) occupies the lateral wall of the 4th ventricle. It contains (20μm) fusiform cells with evenly distributed Nissl substance. There are 2 dendritic trunks, few secondary branches and a paucity of dendritic spines. Dendritic orientation is dorsomedial to ventrolateral. Nucleus magnocellularis medialis (NMM), dorsolateral to NL, contains (15μm) elongate cells, evenly distributed Nissl substance, with 2-4 dendritic trunks, few secondary branches, and a paucity of dendritic spines. Nucleus Angularis (NA), rostrolateral to NL and NMM, have (13-19μm) ovoid soma. There are 2-3 dendritic trunks, some secondary branches and no dendritic spines. NMM and NA have radiate dendritic fields.

The unit response of 18 neurons to tone burst stimuli was examined in 20 animals. Glass micropipettes were hydraulically advanced through the AT. Of 35 neurons studied, a majority show primary-like response patterns in post-stimulus time histograms while interspike interval histograms reveal a high degree of frequency following. These units display low characteristic frequencies (below 1.3 KHZ) with low to moderate rates of spontaneous and driven activity.

17 LOCALIZATION OF SOUND IN SPACE BY MONAURAL ANIMALS. John H. Casseday and William D. Neff. Div. Otolaryngol. and Dept. Psychol., Duke Univ., Durham, N.C. 27710 and Center for Neural Sci., Indiana Univ., Bloomington, Ind. 47401.

Cats with one ear destroyed were trained to localize sound in space in a situation requiring the animals to approach the source of sound in order to obtain food reward. After ablation of auditory cortex ipsilateral to the intact ear, experimental animals were still able to respond correctly. After ablation of auditory cortex contralateral to the intact ear, animals were unable to make the localization discrimination. It is suggested that the auditory pathways contralateral to a given ear carry the information that enables the animal to identify the ear that is stimulated by sound.

18 AFFERENT AUDITORY PATHWAYS INVOLVED IN REFLEX RESPONSES TO ACOUSTIC STIMULI. Joseph C. Chan. Ctr. for Neural Sci., Indiana Univ., Bloomington, Ind. 47401. (SPON: Ilsa Schwartz)

Startle and orienting responses were tested in unesthetized cats before and after ablation of centers or transection of pathways of the auditory nervous system. Both reflex responses were present after unilateral or bilateral ablation of auditory cortex; the orienting response was less accurate. Severe deficit in the orienting response occurred after bilateral transection of the brachium of the inferior colliculus (BIC). Thresholds for startle responses were lower after bilateral transection of BIC.

Unilateral transection of the lateral lemniscus (LL) produced a severe deficit in orienting to sounds in the auditory field contralateral to the side of transection. Bilateral transection of LL abolished the orienting response. Thresholds for startle responses were lower.

Unilateral transection of the ventral acoustic stria affected orienting responses for sounds in the auditory field ipsilateral to side of lesion. Transection of the trapezoid body produced severe deficits in orienting to sounds in both auditory fields and abolished the startle response.

19 CHRONIC INTRACOCHLEAR STIMULATION OF DEAF VOLUNTEERS: PITCH MODULATION AND PARAMETRIC EXPERIMENTS. Donald K. Eddington, Michael G. Mladejovsky*, Derald R. Brackmann* and Wm. H. Dobelle. Neuroprostheses Program, Inst. Biomed. Eng., Univ. of Utah, Salt Lake City, UT 84112 and the Ear Research Institute, Los Angeles, California.

Electrical stimulation of electrodes implanted in the cochlea (inserted through the round window into the scala tympani) of a restricted population of the deaf will produce relatively simple audible perceptions. One bilateral deaf volunteer (congenital) and one unilateral deaf volunteer (aquired) were implanted with multielectrode systems connected to a percutaneous pedestal-connector assembly. Feasibility and methods of chronic implantation with percutaneous leads are presented. The pitch of the perception elicited by electrical stimulation may be varied by varying the electrode being stimulated (place pitch) or by varying the frequency of stimulation at a particular electrode (periodicity pitch). This pitch modulation data along with parametric threshold data and some absolute parameter matching data from the unilateral patient are presented.

20 **WITHDRAWN BY AUTHOR**

21 CORTICO-CORTICAL CONNECTIONS OF THE AUDITORY AREA IN THE OWL MONKEY. K. A. FitzPatrick and T. J. Imig*. Dept. of Neurophysiology, Waisman Center, University of Wisconsin, Madison, Wisc. 53706.

Auditory responsive cortex in the owl monkey consists of six fields which can be distinguished on the basis of cytoarchitecture and physiological mapping experiments (Imig et. al., in press, J. Comp. Neur.). Two of the cortical areas, the primary field A1 and the adjacent rostral field R, comprise the core of auditory cortex. Cytoarchitectonically A1 and R are similar and each contains a complete frequency representation. Surrounding these two areas is an auditory belt region made up of at least four distinct fields, the anterolateral (AL), caudomedial (CM), posterolateral (PL) and rostromedial (RM) areas. In the present experiments injections of tritiated proline were made into A1 and R and the autoradiographic tracing method was used to determine the connections of these two areas with each other and with the surrounding fields. The primary field projects strongly upon R and CM. A moderate projection was found from A1 to RM and PL. Occasionally AL appeared to receive a projection from A1. The rostral field in turn projects strongly upon A1 and PL. R sends a moderate projection to RM and in some cases grains were found in AL following injections into R. Thus A1 and R are reciprocally connected. CM receives a projection only from A1, and PL and RM receive from both A1 and R. AL receives relatively little input from either A1 or R. Both A1 and R give rise to commissural projections. R appears to project upon contralateral auditory cortex through the anterior commissure, while axons of cells in A1 travel to the opposite cortex in the corpus callosum.

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22 EFFECT OF AUDITORY CORTEX ABLATION ON THE LOCALIZATION AND DISCRIMINATION OF BRIEF SOUNDS. Henry Heffner. Bureau of Child Research, University of Kansas and Parsons State Hospital, Parsons, KS. 67357

Dogs with lesions in auditory cortex were tested on their ability first to localize and then to discriminate between brief sounds. In both tests the animals were required to make a spatial response by moving to one of two goal boxes located 60 degrees apart and 8 ft away from a starting position. Though the dogs were unable to localize either a single click or a 0.5 sec burst of clicks emanating from loud-speakers located in the goal boxes, they were able to discriminate the presentation of a single click from silence as well as a 0.3 sec burst of a 100/sec click train from a 0.3 sec burst of a 10/sec click train. The ability of these animals to make a spatial response on the basis of sounds whose sources they could not locate indicates that the observed deficit in sound localization was not due to an inability to make a spatial response to auditory cues in general nor to any general impairment in attention or memory for brief sounds. Instead, these results suggest that the observed deficit is due, perhaps solely, to an impairment in the ability to respond on the basis of the locus of sounds.

(Supported by Grant NICHHD 02528, Bureau of Child Research, University of Kansas)

23 EFFECTS OF ACOUSTIC STRESS ON AUDITORY BRAINSTEM POTENTIALS IN THE C57BL/6J MOUSE. Kenneth R. Henry. Dept. Psychol., University of California, Davis, CA. 95616.

The volume conducted auditory far field response was measured before, immediately after, and two days after a 5 minute exposure to 90 db. white noise. Each component had an immediate increase in latency, and most subjects maintained a partial increase of latency for at least 2 days. The auditory nerve component was more severely affected than were later components, and the latency increase was more pronounced when far fields were obtained at low sound pressure levels (SPL's) than at high SPL's. Brainstem latencies recovered less rapidly in 35 day old mice than in 16 day old subjects, with many younger mice developing recruitment of the central nervous system components. Single gene allelic substitutions at the albino locus also influenced the development and recovery of the far field response.

24 THE PRECEDENCE EFFECT IN YOUNG AND OLD ALBINO RATS. V. Hoeffding* and J.M. Harrison. Dept. Psychol., Boston Univ., Boston, MA 02215.

Control of responding by the location of paired auditory stimuli (precedence effect) was studied in young and old rats. The apparatus was a wire enclosure with two speakers and levers on opposite walls, a response key between the speakers, and a dipper feeder. The stimuli were single pairs of clicks of equal intensity produced by 50 μsec inputs to the speakers, separated by time intervals (Δt's) varying from 15 μsec to 32 msec. On each trial a response on the lever next to the speaker that sounded first was reinforced with food. Degree of control was measured by the percentage of reinforced responses. For short Δt's, the lower threshold (75% reinforced responses) was between 15 and 30 μsec, and the upper threshold was approximately 16 msec. Asymptotic levels of more than 95% were maintained using Δt's between 125 μsec and 4msec. Data for Δt's between 64 msec and 2.048 sec are being collected, as are data for intensity differences comparable to those which would be found between a sound source and a natural echo at a given Δt; the values of Δt at which rats made over 95% correct responses are ones for which naturally occuring intensity differences would be small (averaging less than 5 db).

25 INTERPRETATION OF THE FAR-FIELD ACOUSTIC RESPONSE: A STUDY OF SINGLE NEURONS IN THE BRAINSTEM. C.-M. Huang* and J.S. Buchwald (SPON: R.J. Norman). Dept. Physiol. BRI, Mental Retard. Res. Ctr., Univ. of Calif., Los Angeles, Calif., 90024.

Single units in the brainstem auditory nuclei were studied in order to specify further the anatomy and electrophysiology of cell groups which contribute to the amplitudes of vertex recorded short latency "far-field" acoustic evoked responses. In the cochlear nucleus of the cat, a major subpopulation of short latency (2.5 $\pm$ 0.2 ms) cells was found in the ventral cochlear nucleus with precisely the same response latency to acoustic stimuli as that of the vertex short latency acoustic response component 2. Response patterns of these cells within the first 20 ms were highly predictable from one trial to the next, unlike other cells in the ventral and dorsal cochlear nucleus. This type of cell also showed response characteristics, i.e., changes in response latency and response magnitude, which closely paralleled those of the far-field acoustic response in terms of effects of stimulus repetition rate, stimulus intensity and stimulus frequency. These data suggest that such short latency neurons in the ventral cochlear nucleus may be the major source of far-field response component 2. This functional cell type, with a response latency which corresponds precisely to a particular component of the vertex recorded short latency responses, is presently being studied in other brainstem auditory nuclei up to and including the inferior colliculus. The electrophysiology of such brainstem neurons suggests that within the classical auditory pathway there is a subsystem of fast-conducting cells whose sequential activation is reflected by the far-field vertex responses. (This work was supported by USPHS Grant NS 05437).

26 RELATIONSHIP BETWEEN BINAURAL INTERACTION COLUMNS AND COMMISSURAL CONNECTIONS OF THE PRIMARY AUDITORY CORTICAL FIELDS (AI) IN THE CAT. T. J. Imig* and J. F. Brugge. Dept. Neurophysiol. and Waisman Ctr. on Mental Retardation, Univ. Wis., Madison, WI 53706.

Tritiated [^{3}H]-proline or horseradish peroxidase (HRP) was injected into the high-frequency representation of AI. Within AI opposite the hemisphere receiving large injections of HRP, labeled cells group in clusters in layer III. A few labeled cells are located deep in the cortex. As the result of large injections of [^{3}H]-proline into AI, there appears in sections prepared for autoradiography a dense accumulation of silver grains over the caudal one third of the corpus callosum extending to within about 2mm of its tip. Silver grains are not evenly distributed over AI contralateral to the injection site. They aggregate into a series of vertical bands oriented perpendicular to the cortical surface. Except for a narrow band at the IV-V border, layers IV and V are sparsely labeled compared to the other laminae. Neurons within this region of AI are sensitive to binaural stimulation; a single type of binaural interaction predominates among neurons located within a vertical column. Binaural interaction columns were mapped in AI of the hemisphere opposite the one receiving injection of HRP or [^{3}H]-proline. Borders of physiological columns correspond closely with borders of vertical columns formed by silver grains in autoradiographs. Columns characterized by cells whose responses to binaural stimulation are greater than responses to stimulation of either ear alone are relatively densely populated with silver grains and HRP-positive cells. Columns characterized by neurons whose responses to contralateral stimulation are suppressed by stimulation of the ipsilateral ear are relatively devoid of silver grains and HRP-positive cells. (NS05326)

27 ELECTROPHYSIOLOGICAL PROPERTIES OF MIDDLE EAR AND LARYNGEAL MUSCLES IN LITTLE BROWN BATS. Philip H-S. Jen and Nubuo Suga* Division of Biological Sciences, University of Missouri, Columbia, Missouri 65201 and Department of Biology, Washington University, St. Louis, Missouri 63130.

In little brown bats (Myotis lucifugus), both the middle-ear and laryngeal muscles are highly developed. The large middle-ear muscles are powerful in controlling the transmission of the sound energy across the ossicular chain and the hypertrophied laryngeal muscle is related with the emission of ultrasonic signals. When the bat emits orientation sounds, action potentials of middle-ear muscles appear approximately 3 milliseconds after those of the laryngeal muscle; this activity of middle-ear muscles attenuates the vocal self-stimulation and improves the performance of the echolocation system. When an acoustic stimulus is delivered, both types of muscles contract; action potentials of laryngeal muscles appear approximately 3 milliseconds after those of the middle-ear muscles. The activity of acoustic laryngeal muscle reflex may act as a negative feedback loop to stabilize the operation of the vocalization system. Since these two groups of muscles discharge action potentials prior to the vocalization as well as during the acoustic stimulus, both muscles are apparently activated in a coordinated manner not only by the nerve impulses from the vocalization system, but also by those from the auditory system. (Work supported by NSF).

28 ASCENDING AUDITORY PATHWAYS TO INFERIOR COLLICULUS IN THE TREE SHREW. Doyle R. Jones* (SPON: James Coleman). Depts. Psych., and Surgery, Duke Univ., Durham, N.C. 27706

In order to determine whether projections of the subdivisions of the cochlear nucleus (CN) and superior olivary complex (SOC) converge or remain separate at the central nucleus of the inferior colliculus (ICc), small injections of horseradish peroxidase were made in the ICc of the tree shrew. After injections in the center of ICc, labeled cells were found in medial superior olive (MSO) ipsilateral to the injection, lateral superior olive bilaterally, dorsal CN bilaterally, and ventral CN contralateral to the injection. After injections at the borders of ICc, only some subdivisions within CN and SOC contained labeled cells. This result suggests that some of these subdivisions have targets in ICc that do not overlap with the targets of other subdivisions. Further evidence shows that targets of other subdivisions do overlap. For example, the presence of labeled cells in MSO was always accompanied by the presence of labeled cells in anteroventral CN; the absence of labeled cells in MSO was always accompanied by the absence of labeled cells in anteroventral CN. This evidence suggests that these two subdivisions have a common target in ICc.

(This research was supported by NIH Grant NS 12322)

29 INPUTS TO CAT COCHLEAR NUCLEUS SEEN WITH HORSERADISH PEROXIDASE (HRP). Eileen S. Kane. Dept. Anat., Univ. of Chicago, Chicago, Ill. 60637.

HRP was injected unilaterally into the caudal cochlear nuclei of over 20 adult cats. After 12-72 hours, cats were intracardially perfused with weak aldehydes, brains removed, and brain stem blocks infiltrated with 30% sucrose. Frozen sections (40μm), incubated in 1-2% diaminobenzidine, were studied for granule-filled neurons in other auditory areas, other cochlear nucleus regions, and other brain stem nuclei (control areas). Cases of most interest involved injections of just the octopus cell area (OCA) or just the dorsal cochlear nucleus (DCN). After the former injections, medium-sized (about 15-20μm), ovoid neurons of ipsilateral dorsal and ventral lateral periolivary areas of the superior olivary complex (SOC) contained reaction product. Contralaterally, multipolar cells of dorsomedial periolivary areas stained. No reacting neurons were seen in the main SOC nuclei of either side, or in higher auditory nuclei after just OCA injections; axons of the trapezoid body (TB) were stained. OCA injections also showed projections from both anterior regions of the cochlear nucleus and from DCN. After DCN injections, neurons of the dorsal and ventral nuclei of the lateral lemniscus reacted bilaterally. These cells were about 20-25μm in length; some looked multipolar. Occasional cells of the contralateral inferior colliculus contained granules. No cells of the SOC reacted after DCN injections. In combined injections of OCA and DCN, a summation of reacting neurons appeared (periolivary cells, nuclei of lateral lemniscus and posterior colliculus). Injections of more anterior PVCN regions showed reacting cells in the hilus of the lateral SOC nucleus. Thus, our results with HRP confirmed lesion studies that deep DCN and OCA receive scanty and fairly proximal descending projections. (Supported by Deafness Res. Fdn. and USPHS Grants NS 12071 and NS 00008 (RCDA) to the author).

30 PROJECTIONS OF FIELD L IN THE CANARY, Serinus canarius - AN AUTORADIOGRAPHIC STUDY. Darcy B. Kelley and Fernando Nottebohm.
The Rockefeller University, New York, N.Y. 10021

Auditory experience plays an important role in the vocal development of oscine songbirds. While we have recently demonstrated an efferent CNS pathway controlling song in the canary (Nottebohm et al., 1976), the anatomical connections with afferent auditory areas have not been established. Projections of an auditory telencephalic area, field L of Rose, were therefore investigated using the technique of amino acid autoradiography. Five to 20 nanoliters of tritiated leucine (100 μCi/μl) were injected stereotaxically into field L and the adjacent neostriatum using a tapered microsyringe or glass micropipette. Birds were sacrificed after 24 or 48 hours, perfused, the brains embedded in paraffin and processed using standard autoradiographic techniques. Slides were exposed for 1, 4 or 12 weeks. Injection sites did not invade the caudal hyperstriatum or paleostriatum. All projections noted to date have been ipsilateral. Immediately caudal to the injection site, light labelling of nucleus ovoidalis was seen. Label was also seen in a restricted zone at the medial and ventral borders of the hyperstriatum ventrale pars caudale (HV_c), posterior to field L. More caudally, labelled fibers turned ventrally to travel through the archistriatum, collecting in a compact bundle among the medial fibers of the tractus occipitomesencephalicus. These labelled fibers could be seen outlining the anterior and ventral borders of nucleus robustus archistriatalis (RA). Nucleus ovoidalis is the thalamic auditory relay nucleus to field L. Both HV_c and RA are involved in the motor control of canary song.
(Supported by NIH grant MH-28083).

31 **CELLULAR LOCALIZATION OF ADENYLATE CYCLASE ACTIVITY IN THE GUINEA PIG COCHLEA.** Thomas P. Kerr* and Jochen Schacht. Kresge Hearing Research Institute, University of Michigan Medical School, Ann Arbor, MI. 48109.

Structures of the cochlear duct were isolated by low-temperature microdissection, and then incubated with the specific substrate adenylyl imidodiphosphate (AMP-PNP) to demonstrate its enzymatic conversion to cyclic AMP and imidodiphosphate (PNP). For biochemical analysis, cochlear tissues were separated into: a) organ of Corti and spiral limbus; b) lateral wall (stria vascularis, spiral prominence and spiral ligament); and c) myelinated axons of the cochlear nerve taken from the modiolus. Cyclic AMP formed during incubation of homogenates was assayed by protein binding. Highest activity was found in the cochlear nerve and the Corti/limbus preparations, and the enzyme was present also in the lateral wall.

Initial incubations for cytochemical localization of adenylate cyclase were performed with glutaraldehyde (G) prefixation, and lead ion (Pb^{++}) in the incubation medium (Howell and Whitfield, J. Histochem. Cytochem. 20, 873, 1972) to form a Pb^{++}-PNP reaction product visible under the electron microscope. With this procedure, reaction product was restricted to epithelial cells of the spiral limbus and inner sulcus. Since G and Pb^{++} may cause enzyme inhibition, other incubations were performed using mild formaldehyde (F) fixation, and strontium ion (Sr^{++}) in place of Pb^{++} (Ernst, J. Histochem. Cytochem. 20, 13 and 23, 1972). In F/Pb^{++} and F/Sr^{++} preparations, a wider distribution of reaction product was found: it extended to the sensory and supporting cells on the hair-bearing surface of the organ of Corti, and to the spiral vessels beneath it. In the lateral wall, reaction product was visualized on marginal and basal cells of stria vascularis, and on spiral prominence epithelium, as well as in the capillaries of the spiral prominence and ligament. (Supported by NIH Program Project Grant No. 05785 and NIEHS contract No. NO.-ES-2-2110.)

32 SPATIOTEMPORAL PATTERNS OF PRIMARY AND DISTORTION COMPONENTS OF COCHLEAR RESPONSES TO PHASE-LOCKED TWO TONES. D.O. Kim and C.E. Molnar*, Washington University, St. Louis, Mo. 63110.

Using phase-locked two tones f_1 and f_2 ($f_1<f_2$), spatiotemporal patterns of cochlear responses at primary stimulus frequencies and at intermodulation distortion frequencies are measured from Fourier series representations of period histograms of many (up to 340) single cochlear nerve fibers of various characteristic frequencies in individual cats. Spatial patterns of two-tone suppression show that f_1 component is suppressed over a region with strong f_2 component, and vice versa. We have observed that the distortion products (f_2-f_1) and ($2f_1-f_2$) are most prominent and display spatial distributions of both amplitude and phase which are very similar to those of an externally applied single tone of the same frequency over a region near the characteristic place involved. This similarity strongly suggests that the distortion products are mechanically propagated along the basilar membrane. With $f_2/f_1 = 3/2$ so that $(f_2-f_1) = (2f_1-f_2) = 0.5f_1$, we have observed that both the amplitude and phase of the distortion product at $0.5f_1$ go through pronounced variations with changes in the input phase relationship, analogous to the psychophysical observations of Hall [J. Acoust. Soc. Amer. 51: p 1872]. This monaural phase effect with two frequencies is interpreted to be interactions of mechanically present (f_2-f_1) and ($2f_1-f_2$) components whose amplitudes are comparable but with phases changing in opposite direction with the input phase variation.

33 TOPOGRAPHICAL FREQUENCY REPRESENTATION IN THE TELEOSTEAN MIDBRAIN.
Eric. I. Knudsen. Dept. Neurosci., Sch. Med., UCSD, San Diego, CA 92093

A prominent feature of neurophysiological organization in the midbrain acousticolateral area of mammals, birds and reptiles is the orderly progression of unit tuning properties with dorsoventral unit depth. A single unit study on the midbrain acousticolateral area (the torus semicircularis) in catfish revealed an analogous functional organization.

A major input to the torus semicircularis arises from a subpopulation of lateral-line receptors, which detect low frequency (<50 Hz) electric fields. These electroreceptors are distributed over the entire body surface and exhibit relatively uniform frequency tuning to 3-7 Hz fields. Interestingly, electroreceptive units in the torus semicircularis are tuned to various frequencies ranging from 0.5 to 15 Hz fields. In this study frequency tuning properties were correlated with unit depth. Frequency tuning was measured in terms of frequency-threshold and best frequency (the frequency to which the unit responds with the greatest number of spikes). Unit depth was measured from the dorsal surface of the torus semicircularis. Frequency-threshold curves show a gradual transition from low pass (10 db cutoff = 3-10 Hz) to band pass (centered at 8-15 Hz) types with dorsoventral depth. Analysis of unit best frequencies corroborates these data. Electrode tracks made at the optimal angle of 25° from the sagital plane encountered units which progressed in best frequency at an average rate of 5.5 Hz/100 μm (calculated by a linear regression on a cartesian plot of 48 points, r = .760). Although the correlation of unit tuning with unit depth is perhaps not as strong as it is in higher verbrates, it is significant that such a topographical frequency representation has been generated entirely by neural processing of input from a homogeneous population of receptors, without reference to a spatial separation of frequencies like that provided by the cochlea.

34 HISTOCHEMICAL STAINING FOR GAMMA-AMINOBUTYRIC ACID TRANSAMINASE (GABA-T) IN THE COCHLEAR NUCLEI, SUPERIOR OLIVARY COMPLEX, AND CEREBELLAR CORTEX.
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The hindbrain of guinea pigs was stained for GABA-T with Van Gelder's method (J Neurochem 12: 231, 1965). In the cochlear nuclei, cell bodies with a preponderance of primary auditory input stained heavily. Regions with less primary input, compared to that of their central connections, showed staining mainly in the neuropil. In each cytoarchitectonic subdivision of the ventral cochlear nucleus many somata, corresponding to one or more of the large neuronal types of Brawer et al (J Comp Neur 155: 251, 1974), were stained; little of the neuropil stained except in the external granular layer. In the dorsal cochlear nucleus the neuropil (including the glomeruli) was extensively stained, especially in the molecular layer; some cell bodies stained lightly. In the olivary complex, cell bodies stained heavily in the ventral and medial nuclei of the trapezoid body, moderately in the dorsomedial periolivary nucleus, less so in the lateral superior olive, and not at all in the medial superior olive. The neuropil stained only in the lateral superior olive and the ventral nucleus of the trapezoid body.In the cerebellar cortex there was intense staining of the axonal plexus in regions where putative GABAminergic axons terminate. The present observations suggest that some GABA-T may occur in axonal endings. Comparison of histochemical staining for GABA-T with assays for glutamate decarboxylase (GAD) levels (Wenthold and Morest, Neurosci Abstracts, 1976) suggests that there are relatively high GAD levels in regions of dense neuropil staining. However, high GAD levels can also occur in regions where no GABA-T staining of neuropil or cell bodies was found.

35 DIFFERENTIAL, INTRACOCHLEAR RECORDING OF THE COCHLEAR MICROPHONIC IN PIGEON. Martha G. Pierson* (SPON: M.L. Wiederhold). Northwestern University, Evanston, IL 60201.

Using tone bursts as stimuli, the auditory receptor potential, the cochlear microphonic (CM), was characte ized as a function of stimulus frequency and intensity in the pigeon. A differential electrode technique allowed an isolation of response from a limited portion of the cochlea.

At low input levels, the pigeon's CM was found to exhibit simple linearity as a function of stimulus strength. The low level CM also exhibits fidelity with respect to the middle ear transfer function as a function of frequency. At high input levels the CM becomes nonlinear, exhibiting saturation in response to sufficiently intense signals. And, at higher input levels, the pigeon's CM shows a prominent nonlinearity centered at the characteristic frequency of the electrodes.

These behaviors are largely consistent with the mammalian CM. However within the context of avian auditory function, one facet of these results is particularly surprising. Generally, it is assumed that cochlear and retrocochlear sensitivity is determined by the middle ear. As expected, the CM measured in this study reflects the avian middle ear properties. However, behavioral and neural frequency response functions of the pigeon are inordinately insensitive at low frequencies according to other studies. It is probably not coincidental that the apex (the low frequency region) of the avian cochlear consists exclusively of a primitive type of hair cell, the so-called "tall hair cell" whereas the basal avian cochlea through evolution has added a second type of receptor cell, the "short hair cell." It is suggested that those avian receptor cells which transduce low frequency stimuli (tall hair cells) are physiologically less sensitive than short hair cells.

36 UNIT RESPONSES FROM THE COCHLEAR NUCLEI OF THE RED-WINGED BLACKBIRD. M. B. Sachs and J. M. Sinnott*, Dept. of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, Md. 21205.

Responses to sound stimuli have been recorded from cells in nucleus angularis and nucleus magnocellularis of the anesthetized red-winged blackbird (Agelaius Phoeniceus). This paper will be concerned with responses to pure tone-burst stimuli. Response patterns were characterized by their post stimulus time (PST) histograms. The form of these histograms for one cell could vary with the frequency and/or sound level of the tone bursts. For most cells frequencies near the characteristic frequency produced an increase in discharge rate. Although PST histograms for CF tones were often similar to those of auditory-nerve fibers (i.e., a maintained increase in discharge rate), many cells exhibited more complex patterns, including "on", "pauser", and "buildup" types. Bands of frequencies which caused inhibition of spontaneous activity were often present on either side of CF. Two cells showed inhibitory responses for tones of all frequencies. Electrode locations were determined histologically so that recordings could be associated with either nucleus angularis or magnocellularis. Responses from these nuclei will be compared with those from the mammalian dorsal and ventral cochlear nuclei. [Supported by NIH Grant No. 5 R01 NS 12112 and AFOSR Contract No. F 44620-71-C-0024.]

37 FAR FIELD RECORDING OF THE COCHLEAR MICROPHONIC TO PURE TONES FROM THE SCALP OF ANIMALS. Vincent L. Schwent*and Don L. Jewett. Dept. Ortho. Surg., Sch. Med., UCSF, San Francisco, CA. 94143.

Using a modification to our signal averaging equipment, we have been able to record precise, clearly measurable cochlear microphonic (CM) waveforms from the scalp of cats and rats using skin-placed needle electrodes, continuous tone auditory stimuli, repetition rates up to 6000 sweeps per second and sweep durations as short as 150 μsec. Averaged CM responses have been recorded in the range of tone frequencies from 0.1 KHz to 12 KHz. To establish that the waveforms recorded were CM and not electrical or mechanical artifacts or contaminated with neural responses, a variety of control procedures were performed. Simultaneous recordings from the scalp and directly from the round window produced identical waveforms, even for complex, non-sinusoidal stimuli, thereby establishing the cochlear origin of the scalp recorded potentials. The broad frequency response and the stimulus-intensity: response-amplitude relationship of the scalp recordings were characteristic of the CM and further ruled out neural contamination. The absence of a response when the stimulus conduction pathway to the ear was mechanically blocked and the disappearance of the response with the death of the cochlea provide additional evidence against electrical or mechanical artifacts. This scalp recorded CM could be developed as an objective, frequency-specific measure of cochlear function in intact animals and humans.

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38 ULTRASTRUCTURE OF SYNAPTIC PROFILES IN THE DEVELOPING ORGAN OF CORTI IN CULTURE. G.L.Scott*,H.M.Sobkowicz,B.Bereman*and J.E. Rose* Depts. of Anat., Neurol., and Neurophysiol., Univ. of Wisconsin, Madison, Wis. 53706.

It was reported previously that the organ of Corti of a newborn mouse can be maintained for some time in culture (J.Neurocytol., 1975,4:543). Preservation of the fiber pattern and its further development in culture, observed in silver preparations, suggested an electronmicroscopical examination of the material. The main objective was to determine whether and in what form synaptic contacts are present in a cultured organ of Corti when all its central connections are severed. 23 explants which were maintained in culture from 1 to 22 days were prepared for ultrastructural studies. The neuronal, sensory and supporting cells show a remarkably well preserved structural organization. Nerve endings are usually seen in contact with the hair cells, though in younger cultures their number appears to decrease in the second and third row of outer hair cells. The length of the membrane apposition between a single profile of a nerve fiber and a hair cell varies. Contact between a nerve fiber and a sensory cell may exist in the following forms: 1) apposition of the two membranes without thickening 2) apposition of the membranes accompanied by a localized membrane density on either the presynaptic or the postsynaptic side 3) cleft with asymmetrical membrane thickenings 4) classical synaptic ribbon surrounded by a crown of vesicles. The sites of membrane specializations contain a variety of vesicles which may be present at both the presynaptic and the postsynaptic sites, or only at the presynaptic location. Clear-center synaptic vesicles as well as dense core and coated vesicles are present. In addition, large vesicles,in formations that resemble growing tips of nerve fibers, are seen occasionally near the basal portions of the hair cells. To what extent some synaptic profiles develop in culture will be discussed.

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39 **RESPONSE** OF SINGLE AUDITORY NEURONS TO WEAK, PULSED MICROWAVE RADIATION. Ronald L. Seaman* and Robert M. Lebovitz, Dept. of Physiology, University of Texas Health Science Center, Dallas, Texas 75235.

Human observers experience auditory sensations when they are exposed to pulsed microwave radiation (MWR) at average incident power densities much below those permissible under present U.S. safety guidelines. Using cats under barbiturate anesthesia, we have investigated the response of single eighth nerve fibers and of single neurons of the cochlear nuclei to low level pulsed microwave radiation (MWR). MWR at 918 MHz was delivered to the animal via an applicator-antenna oriented towards the posterolateral aspect of the skull. The average incident power density never exceeded 250 $\mu W/cm^2$, whereas the current U.S. safety standard is 10 mW/cm^2. The responses of single units to the MWR were compared with their responses to acoustic click stimuli delivered via a pulse driven earphone inserted in one hollow ear bar of the stereotaxic apparatus. With appropriate controls to preclude electrical or mechanical artifacts and utilizing MWR pulses of 25 $\mu J/cm^2$ or less, a distinct effect on single cells of the auditory system could be demonstrated. Effects on single cell discharge could be observed in response to MWR pulse energies as low as 5 $\mu J/cm^2$. Examination of the acoustic click response of such MWR responsive cells indicated that in most instances an acoustic equivalent to the MWR pulse could be defined. These data suggest that MWR induced audition includes a component derived from displacement of the basilar membrane. Classical mechanical stimulation by bone-conducted acoustic energy derived from the microwave energy seems a likely explanation for this effect, although more complex modes of interaction of the MWR with the inner ear are also suggested. (Supported by DHEW Grant FD-00661 to RML)

40 ADAPTATION, SATURATION, AND DYNAMIC RANGE OF SINGLE AUDITORY NERVE FIBERS. Robert L. Smith. Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

The operating range of the auditory system approaches 120 dB whereas the apparent dynamic range of single auditory nerve fibers is generally below 40 dB. It could be possible to reconcile this discrepancy if the actual operating range of a unit shifted according to its state of adaptation. To test this possibility, responses were obtained from single auditory nerve units of anesthetized Mongolian gerbils and guinea pigs. Signals were tone bursts at the units' characteristic frequencies (CF) and were applied during or after CF adapting tones. The firing rates produced by the signals were averaged over 10- or 40-msec intervals. Adapting durations were up to 300 msec and intensities were within 60 dB of the units' thresholds.

A general finding was that the operating range of a unit was not influenced by adaptation and was always limited by the same peripheral saturation. For example, both adapted and unadapted rate-intensity functions asymptotically approached saturation values over the same range of sound-intensity levels. Adaptation produced only a subtractive shift along the firing-rate coordinate. In addition, the single-unit responses did not behave according to Weber's Law. To explain, when a signal was superimposed on an adapting background, the response to the signal decreased drastically at high background intensities even though the signal energy was increased in proportion to the background energy. Hence, to the extent that auditory information is encoded by changes in the overall firing rate, the short-term adaptation investigated does not produce shifts in sensitivity and extend the operating ranges of auditory nerve fibers.

41 AN EXPERIMENTAL DEMONSTRATION OF POLARITY-OPPOSITION BETWEEN INNER AND OUTER HAIR CELLS IN THE COCHLEA. W. G. Sokolich* and J. J. Zwislocki (SPON: D. A. Robinson). Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

The time-locked responses of auditory-nerve fibers to low-frequency trapezoidal deflections of the basilar membrane contain both velocity and displacement components. The polarities of both response components depend solely, and in a systematic way, upon the fiber's most-sensitive or "characteristic" frequency (CF). The only simple way we could reconcile the observed polarity reversals with the known pattern of afferent innervation and with the homogeneous morphological polarization of the cochlear hair cells was to assume that they resulted from the incomplete cancellation of two opposing inputs. Whereas the input from inner hair cells was assumed to provide excitation (inhibition), the input from outer hair cells was assumed to provide inhibition (excitation) during deflection of the basilar membrane toward scala vestibuli (tympani). To test the polarity-opposition hypothesis we recorded the responses of auditory-nerve fibers to the trapezoidal stimulus in kanamycin-treated Mongolian gerbils. Their cochleas were subsequently examined and found to contain full complements of inner hair cells in some cochlear regions highly depleted of outer hair cells. In accordance with the predictions of the model, the responses of fibers associated with these regions were abnormal and showed unidirectional inhibition (excitation) during short-term sustained displacement and/or motion of the basilar membrane toward scala tympani (vestibuli). Furthermore, the responses of fibers associated with cochlear regions containing full complements of both hair-cell populations were either abnormal and scala-tympani excitatory or were normal.

42 RESPONSE CHARACTERISTICS OF CAT AUDITORY NEURONS LOCATED WITHIN THE DORSAL PERIOLIVARY CELL GROUPS. Chiyeko Tsuchitani, Sensory Sciences Center, Graduate School of Biomedical Sciences, University of Texas Health Sciences Center at Houston, Houston, Texas 77030.

Single unit discharge were recorded extracellularly with stainless steel microelectrodes from auditory units located dorsal to the lateral and medial superior olives. Stimuli consisted of monaurally or binaurally presented tone bursts. The response measures obtained were: effective ear, nature of effect, stimulus frequency representation, maximum output, latency of response, and temporal pattern of discharges. The location of the unit studied or the end of the electrode tract and the MSO cell layer were marked with electrolytic deposits of iron from the recording electrode. Following an experiment the locations of units studied were determined histologically. Most of the periolivary units located dorsal to the lateral superior olive (the dorsal and dorsolateral periolivary nuclei) were excited by stimulation of the ipsilateral ear and unaffected by stimulation of the contralateral ear. These units were narrowly tuned and produced low discharge rates. They responded with long latencies to tone burst stimuli and exhibited large shifts in latency with increases in stimulus level. They produced chopper type PST histograms to CF tone burst stimuli that had wide peaks and long interpeak periods. Many of these sustained chopper PST histograms also contained a pause period between the first and second peaks. Most units located dorsal to the medial superior olive (dorsomedial periolivary nucleus) were affected by stimulation of the contralateral ear, while fewer were affected by stimulation of the ipsilateral ear. The responses elicited by contralateral stimulation were varied. These units tend to be widely tuned and respond with long latencies. Supported by NINCDS Research Grant.

43 TRANSMITTER RELATED ENZYMES IN THE GUINEA PIG COCHLEAR NUCLEUS. R. J. Wenthold and D. K. Morest. Laboratory of Neuro-otolaryngology, NINCDS, NIH, Bethesda, Md. 20014.

The guinea pig cochlear nucleus and auditory nerve were analyzed for choline acetyltransferase (ChAc), glutamate decarboxylase (GAD), and tyrosine hydroxylase (TH). The auditory nerve was low in all three enzymes with ChAc = 1.7 pmoles acetylcholine formed/min/mg protein, GAD = 0.08 nmoles GABA formed/min/mg protein and TH = 1.0 pmoles DOPA formed/min/mg protein. Specific activities of ChAc, GAD and TH were determined for whole cochlear nucleus after unilateral cochlear ablation. Enzyme levels did not decrease significantly in the cochlear nucleus on the lesioned side compared to the unlesioned side at two, four or six weeks after the lesion was made. Glutamic acid and aspartic acid decreased in the cochlear nucleus after cochlear ablation. For analyzing the distribution of ChAc and GAD within the cochlear nucleus, anesthetized animals were perfused with cold 0.12M sodium phosphate (pH 7.3) and the brain stem removed and sectioned at 200μm. Cytoarchitectonic subdivisions corresponding to those described by Brawer et al. (J. Comp. Neur. 155: 251, 1974) were dissected at 4°C, homogenized and assayed for ChAc, GAD and protein. ChAc and GAD were distributed throughout the cochlear nucleus with highest specific activity for ChAc in the granular layer of the anteroventral cochlear nucleus and for GAD in the molecular-fusiform layers of the dorsal cochlear nucleus. The data suggest it is unlikely that acetylcholine, GABA or a catecholamine is a transmitter between the auditory nerve and the cochlear nucleus. The distribution patterns of ChAc and GAD in the cochlear nucleus may be related to specific types of axons projecting from central regions.

44 RELATIONSHIP OF ACOUSTIC INTENSITY TO AUDITORY RESPONSE CHARACTERISTICS OF NEURONS IN THE MOUSE INFERIOR COLLICULUS. James F. Willott, Leo M. Chalupa, and Kenneth R. Henry. Dept. Psychol., UC Davis, CA. 95616.

Extracellular recordings were obtained from single units in the central (ventrolateral) nuclei of the inferior colliculi of lightly anesthetized C57BL/6J mice. Pure tones and white noise bursts were presented to the contralateral ear in a calibrated closed system. Frequency specificity and tonotopic organization were determined, but particular attention was given to response properties as a function of stimulus intensity (I). Input-output (intensity-spike count) functions of 3 notable types were observed: 1. Discharge rates increased monotonically for considerable increases (up to 50 dB) in suprathreshold I before levelling-off or decreasing; 2. Discharge rates were unaffected over broad ranges of suprathreshold I (up to 50 dB); 3. Maximum discharge rates were reached within 5 dB of threshold before levelling-off or decreasing. Some units of the latter type were remarkably sensitive to small I changes, going from no evoked activity to vigorous responding with increments of 1-3 dB. Different frequencies often resulted in differently shaped input-output functions for the same unit.

PST histograms revealed several types of discharge patterns which have been observed in other species: transient onset, off, "primarylike" sustained, and onset-"pause"-sustained. The effects of acoustic I upon discharge patterns were of 2 kinds. 1. The same pattern was maintained for a broad I range, and the overall rate changed in accord with one of the 3 types of input-output functions. 2. The response pattern changed from one type to another as a function of I. This often occurred when short latency (onset) and longer latency components of the discharge pattern varied independently as I was varied, with the 2 components simultaneously showing different types of input-output functions.

45 PROJECTIONS TO THE INFERIOR COLLICULUS IN THE ECHOLOCATING BAT, Pteronotus parnellii parnellii. John M. Zook* and J. H. Casseday. Depts. of Psychology and Surgery, Duke U., Durham, N. C.

In echolocating species of bats, auditory areas of the brain are unusually hypertrophied and often distorted, so that it is difficult to recognize which structures are homologous to auditory structures in other mammals. In this study auditory areas of lower brain stem and cortex of P. p. parnellii were identified by the method of retrograde transport of horseradish peroxidase (HRP). Small amounts (.01 to .02 µl) of HRP were injected into the inferior colliculus via micropipettes. In the superior olivary complex three conspicuous nuclei were seen: a lateral nucleus which contained labeled cells bilaterally, a dorsomedial nucleus which contained labeled cells ipsilateral to the injection, and a medial nucleus within the trapezoid body which did not contain labeled cells. Comparison with results after injection of HRP in inferior colliculus in other mammals suggests the hypothesis that these nuclei are homologous to lateral superior olive, medial superior olive, and medial nucleus of the trapezoid body. Cells in the dorsal part of the lateral lemniscus were labeled bilaterally; cells in the ventral part, ipsilaterally. Labeled cells were found in all three subdivisions of the cochlear nucleus contralateral to the injection site. Labeled cells in the cortex were restricted to layer V and were found in a relatively large, although circumscribed, area of temporal cortex ipsilateral to the injection. Single units which respond to sound have been found in a closely corresponding area of cortex in the bat, Myotis lucifugus (Suga, J. Physiol., 181: 673, 1965). Supported by NIH grant NS 12322.

Axoplasmic Transport

46 NEURONAL UPTAKE OF PEROXIDASE INJECTED INTO BLOOD OR CEREBRAL VENTRICLES.
Richard D. Broadwell and M. W. Brightman*. NIH, Bethesda, MD 20014.

After intravenous injection or ventriculo-cisternal perfusion of horseradish peroxidase (HRP) in mice, uptake of the protein by intact terminals versus undamaged neuronal perikarya and the subsequent fate of the HRP within the cell have been investigated by light and electron microscopy. Within 2 hrs. of vascular infusion, HRP crosses permeable capillaries in the median eminence, neurohypophysis and area postrema to fill the extracellular clefts in and around the hypothalamic arcuate and ventromedial nuclei and the X and XII cranial nerve nuclei in the medulla. Neurosecretory axon terminals in the neurohypophysis and median eminence incorporate HRP into pinocytotic and synaptic vesicles. Profiles of smooth endoplasmic reticulum in a few terminals likewise contain peroxidase. By 8-12 hrs. post-injection, somata in the hypothalamic neurosecretory arcuate, supraoptic and paraventricular nuclei and in brain stem cranial nerve nuclei contain HRP-positive granules, presumably as a result of retrograde axoplasmic transport of the protein from their axon terminals. Labeling of somata in the XII and arcuate nuclei is not as evident in electron micrographs. In thin plastic sections, perikarya in the hypoglossal nucleus of non-injected, control mice contain an average of 20 dense bodies per section. Thus, the only certain sign of HRP-labeling of cell bodies is not the many pre-existing dense bodies but rather bits of dense, smooth endoplasmic reticulum and vacuoles or multivesicular bodies rimmed with reaction product. Almost invariably, labeled multivesicular bodies are close to or continuous with labeled tubular profiles of endoplasmic reticulum.

In order to assess the fate of HRP within the cell body of a damaged neuron, peroxidase was injected intravenously and 12 hrs. later, when both hypoglossal nuclei are densely labeled, one hypoglossal nerve was ligated. Twelve additional hours after ligation the brains were fixed. The somata on the ligated side contain far fewer HRP granules than do those on the opposite, uninjured side; therefore, in neuronal somata with crushed axons there is a heightened enzymatic degradation of HRP. Any protein that may have been pinocytosed directly by these somata would also be expected to be rapidly degraded. To determine whether appreciable amounts of protein are incorporated directly by undamaged cell bodies, 0.01-0.04ml of a 10% solution of HRP was perfused into a lateral cerebral ventricle. Four hours later, extracellular clefts throughout the brain contain HRP reaction product. When viewed with dark field microscopy somata of the X nucleus appear labeled but those of the XII nucleus contain few if any granules that exceed those in uninjected controls. In electronmicrographs, only a few vacuoles and multivesicular bodies in somata of the XII nucleus are HRP positive. By 12-24 hrs. post-injection, all perikarya in the brain, excluding those of the cranial motor nerve nuclei, are labeled. The scant labeling of somata in hypoglossal and possibly in other cranial motoneurons after ventricular infusion may be due to a low level of endocytosis, a rapid enzymatic degradation, and the anterograde transport of protein out of the cell body. Somata of the X nerve nucleus are more heavily labeled at 12 hrs. than at 4 hrs. Conceivably, axon collaterals from the X nerve may project to the area postrema and would therefore be bathed by HRP diffusing from the fourth ventricle. These perikarya would then receive most of their protein from terminals rather than by cell body uptake. The above results suggest that even hours after undamaged neuronal perikarya are exposed to the amount and concentration of peroxidase used, they pinocytose only small amounts of the protein.

47 SLOW AXONAL FLOW: RATE OF TRANSPORT, DISTRIBUTION ALONG THE AXON AND IN SUBCELLULAR FRACTIONS. Paul Cancalon and Lloyd M. Beidler, Dept. of Biol. Science, Florida State Univ., Tallahassee, Fla. 32306

Most of the work done on the rate and composition of slow axonal flow has been performed on the material arriving at the synapses or in branched and myelinated nerves. The garfish olfactory nerve with up to 30 cm. (200 mg.) of unbranched unmyelinated nerve is particularly well suited for the study of the distribution of radioactivity along the axon and in various subcellular nerve fractions.

Profiles of TCA insoluble transported radioactivity were determined at 21°C between 8 and 110 days after application of ^{3}H leucine to the olfactory epithelium. The maximum of a well defined peak of TCA insoluble material moves along the nerve at a rate of 0.92±0.02 mm/day. Apparent rates of transport were also measured for the base of the leading edge of the peak and the base of the back of the peak. Values of 1.61± 0.05 and 0.25 ± 0.04 mm/day were found respectively. The peak of radioactivity is relatively symmetrical and decreases in height and broadens as it moves along the axon. Labeling in front of the moving peak is significant and at 90 days represents approximately 1/3 of the peak maximum. This radioactivity probably represents material previously deposited in the axon by fast transport, since systemic labeling in the contralateral nerve is only 1/20 of the peak value at 90 days. Radioactivity behind the peak decreases to a value almost as low as in the region ahead of the peak. These results indicate that although the peak broadens as it migrates, there is very little radioactivity deposited in the axon behind the peak.

Double labeling experiments showed that the amount of radioactivity slowly transported was 6.2 ± 0.5 times larger than the amount rapidly transported. Striking differences were also found between the composition of fast transport and slow flow. Study shows the subcellular distribution of slowly moving radioactivity to be: soluble 39.6 ± 4.3%; total membrane 32.0 ± 2.9%; mitochondrial pellet 26.9 ± 2.9%. Previously published values for fast transport were respectively: 13.9 ± 0.8%; 64.7 ± 3.0%; 17.3 ± 0.9% (Brain Res. 89:225-244, 1975). SDS gel electrophoretic profiles of slowly transported proteins were also significantly different from those previously determined for fast transport. In fast transport 60 to 65% of the membranous radioactivity is associated with polypeptides of a MW superior to 54,000 with a major peak (12%) at 126,000 MW. In slow flow, on the contrary, radioactivity is associated with low and intermediate molecular weight polypeptides (more than 80% below 80,000 MW) in both soluble and membranous fractions. (Supported by NIH grant NS 05258).

48 THE USE OF TRITIATED HORSERADISH PEROXIDASE FOR DEFINING NEURONAL PATHWAYS: A NEW APPLICATION. E.E. Geisert Jr.*, (SPON: B.V. Updyke). Neurosciences Training Program and Dept. of Anatomy, Univ. of Wisconsin, Madison, WI. 53706.

In this study, tritiated Horseradish peroxidase (3H-HRP) was used either as a substitute for Horseradish peroxidase (HRP) or in conjunction with HRP in a dual label experiment designed to demonstrate collateral branching systems. In five hemispheres of three cats, the visual cortex (areas 17, 18 or 19) was injected with 2% 3H-HRP, 20% HRP or a mixture of the two. The 3H-HRP was transported from the injection site in the anterograde direction, as demonstrated by the labelling in the cortical terminal field in the superficial layers of the superior colliculus. The 3H-HRP was also transported in the retrograde direction, labelling the cell bodies of neurons in the dorsal lateral geniculate nucleus. When the 3H-HRP was injected in a weak (2%) solution it was not detectable by its enzymatic activity but was detectable by autoradiography. Thus, we have two independent markers for studying axonal systems that branch; 20% HRP (detectable only by its enzymatic activity) and 2% 3H-HRP (detectable only by the autoradiographic method). In the right hemisphere of animal 39, area 17 was injected with HRP and area 18 was injected with 3H-HRP. In the tissue processed by both the autoradiographic method and the HRP staining procedure, two overlapping columns of labelled cells were seen in the right lateral geniculate nucleus. In the region of non-overlap only one type of label was seen, either brown granules in the cells of the lateral portion of the nucleus or silver grains over the cells in the more medial portion of the nucleus. Where the two columns overlap cells were seen which were only HRP stained and cells were seen which were only tritium labeled. In addition, a few cells contained the brown granules inside their cell bodies and these cells also had silver grains directly over their cell bodies. Therefore, this experiment confirms that some of the cells in the lateral geniculate nucleus of the cat project to both area 17 and area 18 by a collateral branching system. This 3H-HRP method is more sensitive and more reliable than the HRP method, since the 3H-HRP method is not dependent upon the enzymatic activity of the protein and because the autoradiographic technique can detect small amounts of transported 3H-HRP or 3H-HRP degradation products. Additional advantages of this method are that it does not restrict investigations to frozen sections and that it allows for the double labelling experiment.

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49 AXONAL TRANSLOCATION OF ^{45}Ca: RELATION TO FAST AXONAL TRANSPORT OF PROTEIN. R. Hammerschlag*, A.Y. Chiu*, B. Weiss* and A.R. Dravid* (Spon: J.E. Burton). Div. of Neurosciences, City of Hope Nat. Med. Ctr. Duarte, CA 91010, and Bio. Med. Res. Div., Sandoz, Ltd., Basel Switzerland.

Axonal translocation of ^{45}Ca has been demonstrated in a variety of in vivo and in vitro preparations at rates similar to those observed for fast axonal transport of protein. We have been interested in determining whether this mobile ^{45}Ca may reflect a role of calcium ions in the coupling of fast-transported proteins to the transport system (Science 188: 273, 1975). Axonal transport of ^{45}Ca was followed in vitro in the 8th & 9th spinal-sciatic nerve complex of bullfrog. Dorsal root ganglia were selectively exposed for 1h at 18° to normal incubation medium with $CaCl_2$ (1.8 mM) replaced by $^{45}CaCl_2$ (600 μCi/ml; 15-30 mCi/mg). Following this initial pulse, exposure of nerve trunks to calcium-free medium (while maintaining the ganglia in normal medium) had no obvious effect on either the rate or the amount of ^{45}Ca transport during a 20h incubation period. (Similar results have been reported for axonal transport of [^{3}H]protein). This selective incubation in calcium-free medium was found to reduce endogenous calcium levels in the nerve trunks by 75-80%. ^{45}Ca transport was also unaffected by selective exposure of nerve trunks to high calcium medium (9 mM) during 20h, conditions that led to a several-fold increase in endogenous calcium levels. Conditions that increased calcium uptake into the ganglia (substitution of LiCl for NaCl during the 1h pulse) increased the amount of ^{45}Ca transported along the nerve trunks. Thus, while ^{45}Ca readily enters a "transportable" calcium pool within the cell soma, once it begins its axonal migration it appears to become sequestered in a pool that is not in ready equilibrium with the major compartment(s) of calcium in the nerve trunk. In double-label experiments addition of cobalt (0.0045 to 0.18 mM) to normal medium bathing both ganglia and nerve trunks suppressed the amount of ^{45}Ca transport in parallel with the amount of [^{3}H]protein transport in a log dose-response manner. Thus, cobalt ions may inhibit an initial calcium-mediated coupling of proteins to the transport system within the cell somata. These results also suggest that the mobile ^{45}Ca is associated with all fast-transported proteins rather than with a specific fast-transported calcium-binding protein(s). If the translocation of ^{45}Ca was a result solely of its transport via a specific carrier protein, cobalt ions might still depress the amount of ^{45}Ca undergoing transport but would not be expected to result in the observed parallel depression of total [^{3}H]protein transport. When ^{45}Ca was allowed to enter the sciatic nerve trunk during a 16h incubation period and ligatures were than placed on the 8th & 9th spinal nerves, further incubation during 8-30h resulted in accumulations of ^{45}Ca both proximal and distal to the ligatures. The ratio of the build-ups indicates that transport of ^{45}Ca in the retrograde direction occurred at a slower rate than in the anterograde direction, similar to the relative rates observed for the bidirectional fast transport of proteins. The present results are consistent with our proposal that calcium may function in the transport mechanism as an ionic bridge during both the loading phase and the translocation phase of fast axonal transport. (Supported by NSF Grant BNS75-17640.)

50 DESIGN AND CHARACTERIZATION OF FLUOROCHROME-CONJUGATED HORSERADISH PEROXIDASES FOR NEURONAL TRACING. J.S. Hanker, J.J. Norden, R.W. Oppenheim, and I.T. Diamond. Dent. Res. Ctr. and Neurobiology Program, UNC at Chapel Hill, NC. 27514, Dept. Psych., Duke Univ., Durham, NC. 27706, and Res. Div., NC Div. of Mental Health Serv., Raleigh, NC. 27611.

Horseradish peroxidase (HRP), although introduced as a general cytochemical tracer, has been particularly valuable in neuronal tracing. Its greater basicity than albumin may contribute to its uptake by a number of different cell types including neurons. However, its peroxidatic activity, responsible for the oxidative polymerization of 3,3'-diaminobenzidine, is probably its most important attribute.

Conjugates of HRP with fluorochromes of different colors (J. Histochem. Cytochem. 24, 609, 1976) could be important in neurobiology for several reasons. They could be useful in the study of topographical organization, such as the somatotopic organization of ganglion cells, as well as in the study of the distribution of axon collaterals from a given neuron. They could also be used to study changes in vascular permeability, the penetrability of glial cell processes, and the phagocytic activity of Schwann cells. If the peroxidatic activity of the HRP were retained in the conjugates, it could be utilized to arbitrate distinction of the tracer from some cellular constituent displaying autofluorescence. The conjugates of HRP with fluorescein isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (TMRITC) were prepared in the usual manner[1]. The FITC conjugates (^{II}HRP-F and ^{VI}HRP-F) were more orange than the buff colored HRP starting materials (Worthington equivalents of ^{II}HRP and ^{VI}HRP) and fluoresced a brilliant yellow. The solid pink TMRITC conjugates (^{II}HRP-R and ^{VI}HRP-R) fluoresced a brilliant mandarin red whereas their solutions had a magenta fluorescence. The conjugates had both fluorescent and peroxidatic activities of an order of magnitude expected of conjugates. Ultraviolet absorption studies suggested that conjugation by covalent bond formation had occurred; a shift in the absorption maximum of ^{VI}HRP from 220 nm to 230 nm, and the absence of the 320 nm peak characteristic of FITC were observed in the ^{VI}HRP-F conjugate.

Unequivocal proof of conjugation through covalent bond formation was obtained by immunoelectrophoresis studies in which each conjugate was compared with its HRP starting material versus rabbit antiperoxidase (from horseradish). The precipitin lines from each conjugate and its HRP starting material were obtained on agar coated slides. When the unstained precipitin lines were exposed to ultraviolet radiation, the line of the TMRITC conjugate fluoresced mandarin red, while the FITC conjugate line had a brilliant yellow fluorescence. The precipitin lines of the unconjugated HRP's, on the other hand, displayed only a very weak autofluorescence. These immunospecific reactions establish that conjugation of HRP with the fluorochromes via covalent bond formation has occurred. (Supported by DE02668, RR05333, MH04849, EY05101, MH16598, and NSF GB31874.)

[1]The conjugates are available from Cappel Laboratories, Inc., Downingtown, PA., 19335.

51 SURVIVAL TIME, ALDEHYDE FIXATION, AND AXONAL TRANSPORT OF HORSERADISH PEROXIDASE. John C. Hedreen, Susan McGrath* and Carolyn Warner*, Dept. of Anatomy, Johns Hopkins Univ. School of Medicine, Baltimore, Md. 21205

Survival studies. After injections of 0.1 µl 30% horseradish peroxidase (HRP) into the caudatoputamen (CP) in rats, survival times of 2,3,4, 6,12,18 hrs., 1,1 1/2,2,3,5, and 8 days, were allowed before perfusion with fixative containing 1.25% glutaraldehyde and 1% formaldehyde. Observations were made on the following phenomena:

Cells with granules indicative of retrograde transport can be seen at 2 hrs. in the parafascicular nucleus and rostral third of the substantia nigra (SN). At 3 hrs. they appear in the remainder of the SN and in the ventral tegmental area, and at 4 hrs. in the dorsal raphe nucleus. From 12 to 24 hours the cells are most numerous and most heavily labeled with HRP. There is some fading apparent at 1 1/2 days, and at 2 and 3 days the granular labelling of cells is much less prominent. At 5 days many fewer cells are identifiable, and none are seen at 8 days.

HRP in presumed axonal terminals in the SN is first seen at 4 hours, and is most prominent at 12 to 24 hrs. Subsequently the terminals fade and are gone at 5 days. At 3 days some are enlarged and appear degenerating. Injury to a neuron is not required to label its terminals since they are seen in the SN in cases with large cortical injections (0.2 µl), presumably because diffusion into CP allows striato-nigral anterograde transport. A larger cortical injection is required to demonstrate terminals in the SN than cells in the SN; thus a higher extracellular concentration of HRP is required for anterograde than retrograde transport.

Visible diffusion of HRP around the injection site was maximal early and thereafter shrank and faded in intensity. At 24 hrs. clear shrinkage of this area had occurred, and by 2 days this region was extremely limited. Clearly the limits of the region from which retrograde transport takes place cannot be defined by examining diffusion from the injection site at a particular survival time. The visible diffusion extended particularly along large blood vessels and bundles of white matter and often entered the rostral thalamus.

Diffuse filling of axons was present in the entire medial half of the internal capsule in a section through posterior thalamus at early survivals. Thus there is an early massive movement of HRP in (and around?) normal and damaged axons. At 18-24 hrs. most of these become quite faded. Those which remain prominently stained may be damaged axons.

Diffusely filled neuronal perikarya are abundant around the injection site to the limits of the early visible diffusion, around large blood vessels below the CP, and around bundles of diffusely filled axons (eg. internal capsule). These cells probably take up HRP locally. Very dark diffusely filled cells occur in many thalamic nuclei. These may be neurons whose axons were damaged by the injection needle. With longer survival the diffusely filled cells acquire granules and the diffuse cytoplasmic HRP fades. At 36 hrs. very few diffuse cells without granules are left, and many cells at the usual borders of early diffusion or near large blood vessels below the CP now have granules and have lost their diffuse HRP content. Clearly cytoplasmic granules are not proof of retrograde transport from terminals.

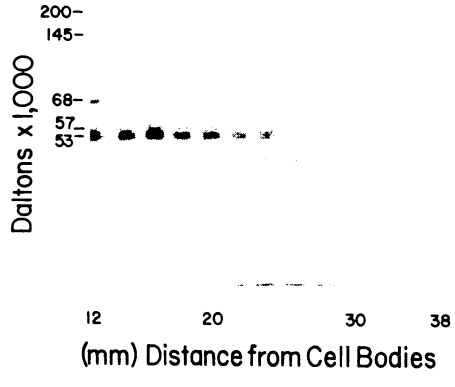
Fixation studies on the effect of glutaraldehyde (G) and formaldehyde (F) on HRP activity in CP injection cases and in somatosensory cortex cases, with 1 day survival, showed that G alone (1.25-5%) is best, and F alone (2-8%) is fair. 0.5% G with 0.4% F allows extensive post mortem diffusion and yields no distant cell labelling. 1.25% G with 1% F is usually good. Unexpectedly, G with F in higher concentrations (eg. 3.5% G with 2% F) strongly inhibits HRP activity. Thus G alone appears to maximally prevent post mortem diffusion, and to lack the inhibitory effect of G and F together.

Supported by NSF GB-41755, NIH NS-10920 and RR-5378.

52 THE AXON AS A BIOLOGICAL CHROMATOGRAPHIC COLUMN. P.N. Hoffman*, M.M. Black* and R.J. Lasek (SPON: J.S. Brodkey). Dept. of Anatomy, Case Western Reserve Univ., Cleveland, Ohio 44106.

The axon lacks the capability for endogenous protein synthesis, and relies on the influx of newly synthesized proteins from the neuron cell body. These proteins are conveyed within the axon by way of the axonal transport system. Using radioactive amino acid precursors it is possible to demonstrate the movement of pulse-labeled neuronal proteins along the axon. This movement occurs at several discrete rates which correspond to the so-called slow (1-2 mm/day), intermediate and fast (400 mm/day) components. Each component studied thus far appears to have a unique protein composition. This finding is consistent with the hypothesis that the different components each represent the transport of different axonal structures. Therefore, using the pulse-labeling paradigm it should be possible to identify the groups of labeled proteins which constitute these distinct axonal structures and organelles. In the case of microtubules, for example, a number of non-tubulin proteins have been identified in preparations of reconstituted microtubules. Are any of these proteins naturally associated with microtubules, or are the associations between tubulin and other proteins artifacts of the isolation procedures? Using the axon as a biological chromatographic column we can identify proteins which are co-transported with tubulin. It is these proteins which may be naturally associated with tubulin as constituents of intact axonal microtubules.

The figure illustrates our ability to identify groups of labeled proteins co-transported at different rates within the axon. It shows a fluro-autoradiograph of an SDS-slab gel which contains the labeled proteins present in the guinea pig hypoglossal nerve 9 days after labeling the hypoglossal nucleus. The labeled proteins present in consecutive, 2 mm long segments of nerve were solubilized in urea, SDS and 2-mercaptoethanol. Two distinct groups of labeled proteins are revealed in this fluro-autoradiograph. One group occupies segments of the nerve extending up to 18 mm from the neuron cell bodies, which are transported at rates up to 2 mm/day. The second group is present at distances of 20-38 mm, and moving at an average rate of 3.2 mm/day. The slower moving group of proteins contains three polypeptides with MWs of 200, 145 and 68,000 d., the slow component triplet. This slower group also contains a protein identified as tubulin (53,000 d.). Data indicates that tubulin and the triplet proteins are capable of independent movement within the axon, suggesting that they represent the movement of different structures. We have hypothesized that the triplet proteins are constituents of axonal neurofilaments (Hoffman and Lasek, J. Cell Biol. 66,351, 1975). We are currently investigating the possibility that non-tubulin proteins are co-transported with tubulin as constituents of axonal microtubules.



53 IMMUNOCHEMICAL ANALYSIS OF THE PROTEINS COMPRISING MYXICOLA (10nm) NEUROFILAMENTS. R.J. Lasek and J.-Y. Wu (Spon: E. Peck). Department of Anatomy, Case Western Reserve U., Cleveland, Ohio 44106 and Department Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

The axoplasm of the giant axon of the marine polycheate, Myxicola is an excellent source of neurofilaments (NF) because NF are the predominant structures in this axoplasm which contains few, if any, microtubules. NF were isolated from homogenized axoplasm by sedimentation through a discontinuous sucrose gradient containing 0.1 M NaCl, 5mM EGTA, 50mM Tris HCl, pH 7.4. Analysis of fractions from the gradient on SDS-polyacrylamide gel electrophoresis showed that the fraction which contained most of the NF also contained two of major bands with molecular weights of 150,000 and 160,000 (Figure). These bands represent more than 90% of the stainable protein on the gel. The gels also showed a number of minor bands, none of which accounted for more than 1-2% of the total protein. Since the 150K and 160K proteins are the major components of Myxicola axoplasm and copurify with NF, these proteins appear to be the primary components of Myxicola NF.

Rabbits were immunized every two weeks with NF protein (NFP) by injection intramuscularly of either 7, 35, or 70 µg of NFP in 0.5 ml saline mixed with 0.5 ml complete Freunds adjuvant. Antibody was detectable in the sera from all of the rabbits two weeks after the 4th injection and a single precipitin line was found with native NFP on immunodiffusion in agar. NFP which was treated with 1% SDS, 5% βME and heated at 90°C for 4 min also produced a single precipitin line. NFP was analyzed by both one dimensional and two dimensional immunoelectrophoresis on SDS-polyacrylamide gels. Two immunoprecipitin arcs formed. One arc corresponded the 150K and 160K bands and the other to minor bands with molecular weights between 47,000 and 60,000. Although SDS-gels indicated that NFP consists of two molecular weight proteins (150K and 160K), the immunoelectrophoretic experiments indicate that these proteins may be composed of lower molecular weight subunits. Alternatively the multiple precipitin arcs seen on immunoelectrophoresis could result, if the NFP antisera contained a series of antibodies against the minor protein bands which are present in NFP. However, this is unlikely, because the antisera which was tested was produced by five injections with 7 µg of NFP, a total of 35 µg. Thus, each of the minor bands which produced precipitin arcs amounted to less than 140 ng of protein per injection. These results may explain the apparent differences in molecular weight which have been found for NFP from various sources. For example bovine NF contain a number of bands on SDS-gels, one of which is similar in its mobility to the 150K of Myxicola. However, the major band in bovine NF has a molecular weight of 51-55K. Possibly Myxicola NF and bovine NF are composed of similar lower molecular weight subunits, but the 160K and 150K proteins of purified Myxicola NF are not as readily dissociated by SDS as those of bovine NF. Further studies with antibody to NFP should assist in the identification of the NFP subunits and allow phylogenetic comparisons of NFP.

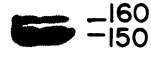


Figure 1. illustrates a SDS-polyacrylamide slab gel of 10 µg of NFP. Only the 150K and 160K bands are visible.

54 CELLULAR UPTAKE AND NEURONAL RETROGRADE TRANSPORT OF FLUOROCHROME-CONJUGATED HORSERADISH PEROXIDASES. J.J. Norden, R.W. Oppenheim, I.T. Diamond, and J.S. Hanker. Dent. Res. Ctr. and Neurobiology Program, UNC at Chapel Hill, NC. 27514, Dept. Psych., Duke Univ., Durham, NC. 27706, and Res. Div., NC Div. of Mental Health Serv., Raleigh, NC. 27611.

Horseradish peroxidase (HRP) has been conjugated to fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate by the thioureido group[1]. The resulting molecules, HRP-F and HRP-R, respectively, fluoresce different colors and their peroxidatic activity or ability to catalyze the oxidative polymerization of 3,3'-diaminobenzidine (DAB) has not been diminished by the conjugation in which the HRP molecules have been covalently bound to the fluorochromes (J. Histochem. Cytochem. 24, 609, 1976).

Studies in different tissues of the mouse and embryonic chick suggest that conjugation of HRP with the different fluorochromes has not affected its uptake or transport by cells. Thus HRP-F or HRP-R can be clearly distinguished by virtue of their fluorescence in phagosomes of kidney proximal convoluted tubular epithelial cells or cuboidal capsular epithelial cells, characteristic of male mice, 15 min after intravenous injection despite the presence of some autofluorescence. The peroxidatic activity of the conjugates could be used to arbitrate any doubtful cases; this activity was observed in kidney phagosomes 5 min after tail vein injections. It was also observed in a few tissue leukocytes near blood vessels in kidney or in very few leukocytes in spleen at this time. Fifteen to twenty min after injection, however, the tracers could be observed in phagocytes of tissue leukocytes throughout the cortex of kidney, in leukocytes in spleen, and in osteoclasts at the surface of remodeling bone. The peroxidatic activity of the conjugates was clearly more discernable than their fluorescence in these cells. The difficulty in detecting the tracers in situ by virtue of their fluorescence could result from some obscure factor such as quenching from some undetermined source, hydrolysis of the thioureido linkage, or fading.

Retrograde transport of the tracers was shown unequivocally by both peroxidatic and fluorescent activity after injection of solutions of the conjugates into the leg musculature of the chick embryo according to the procedure of Oppenheim and Heaton (Brain Res. 98, 291,1975). The conjugates could readily be detected by virtue of fluorescence or DAB oxidation in chick dorsal root ganglion cells or spinal cord α-motoneurons on the ipsilateral side 2 hrs after injection into the hind limb.

Twenty-four hrs after the injection of HRP-F into the foot pad and HRP-R into the thigh of the hind limb of a young mouse, different cells could be observed in lumbosacral spinal ganglia of the ipsilateral side by virtue of the different fluorescent colors. In addition, there is evidence that both conjugates are also transported to the thalamus following injections into cortex. (Supported by DE02668, RR05333, MH04849, EY05101, MH16598, and NSF GB31874.)

[1]The conjugates are available from Cappel Laboratories, Inc., Downingtown, PA., 19335.

55 ANALYSIS OF PROTEINS FLOWING IN DORSAL ROOT AFFERENTS OF RATS. Fredric P. White and Susan R. White. Fac. Med., Memorial University Newfoundland, St. John's, Newfoundland. A1C 5S7

Proteins synthesized by the soma in L4 dorsal root ganglia (DRG) and supplied to the axonal branches extending centrally in the dorsal root (DR) and peripherally in the sciatic nerve (SN) were analyzed for radioactivity following injections of (^{3}H) leucine into the L4 DRG. All experiments were performed on sodium pentobarbital anesthetized Lewis rats. At various times after (^{3}H) leucine injection (3 hours, 2, 3, 4 and 8 days), the rats were again anesthetized and the DR, ventral root (VR), DRG and SN were removed and homogenized in water. The proteins were precipitated by 10% TCA and the precipitate was solubilized in SDS. An aliquot of the solubilized protein was run on 10% acrylamide gels. The gels were then stained with commasic blue, destained, sliced in 1 mm sections and assayed for radioactivity.

The DR always had at least 10 fold more (^{3}H) protein than the VR which consists of axons supplied by soma located in the ventral horn of the spinal cord. Control injections of (^{3}H) leucine over the DR instead of into the DRG resulted in very small but approximately equal amounts of (^{3}H) protein in the DR and VR (at least 10 fold less than in the DR following DRG injection. No changes in distribution of (^{3}H) protein over time were discernable in the DR, VR, SN or DRG following injection over the DR. A similar lack of change over time was found in the VR following DRG injections. However, the DR, DRG and SN gels gave patterns of radioactivity which varied with time between injection into the DRG and removal of tissue. These results indicate that the majority of the (^{3}H) proteins found in the DR and SN after DRG injection are synthesized in the soma and transported to the axonal segments by a flow mechanism.

Distribution over time of (^{3}H) proteins of various molecular weights (MW) often differed in the DR and SN. (^{3}H) proteins with a MW of greater than 250 K daltons represented 5.4 ± 1.3% of the (^{3}H) proteins in the DR at 3 hours, dropped to 1.6 ± .15% at 2 days, rose to 3.8 ± 1.3% at 4 days and dropped again to 1.2 ± .2% at 8 days. On the other hand, in the SN, this group of proteins decreased almost linearly over the 8 day period from a high of 4.3 ± 1.6% at 3 hours to a low of 1.0 ± .03% at 8 days. (^{3}H) proteins in the MW range of 48-72 K daltons peaked in the SN at 2 days. This group rose from 10 ± .9% at 3 hours to 16.6 ± 3% at 2 days and constituted 12.5 ± 2% of the (^{3}H) protein at 8 days. In the DR, this group represented approximately 9% of the (^{3}H) protein in the first 3 days, rose to 12 ± .6% at day 4 and fell to 8.3 ± .6% on day 8. (^{3}H) proteins in the 31-48 K dalton range rose steadily over time in both the DR and the SN. However, a greater amount of this group was present in the SN, and the rate of increase was greater over time in the SN. This group rose from 11.2 ± .9% at 3 hours to 19.2 ± 2.5% at day 8 in the SN, while in the DR, this group increased from 10.1 ± .8% at 3 hours to 12.4 ± 1.1% at day 8. (^{3}H) proteins of less than 15 K daltons constituted 15% of the total (^{3}H) protein in the SN but showed no change over time. In the DR, however, this group rose from 14.0 ± 2.2% to 36.4 ± 2.6% over the 8 days. The DR and SN afferent axonal branches, therefore, appear to be receiving proteins at different rates and in different relative amounts from the soma located in the DRG. (supported by MRC grant MA-5404.

56 RETROGRADE AXONAL TRANSPORT OF ADENOSINE DERIVATIVES IN THE CENTRAL NERVOUS SYSTEM. S. P. Wise* (SPON: Nancy Berman) Dept. Anat. and Neurobiol., Sch. Med., Washington Univ., St. Louis, Mo. 63110

It has been suggested (Schubert and Kreutzberg, Brain Research, 90: 319, 1975) that the nucleoside, adenosine, or its derivatives may be transported by anterograde axoplasmic flow from neuronal somata to synaptic terminals and thence to the somata of postsynaptic cells. The following experiments in birds and mammals indicate that the material may also be transported in the retrograde direction.

Injections of [2,8-^{3}H] adenosine containing 250 μCi in 4-6 μl of saline solution were injected into one eye or into the extraocular musculature of 7-9 day old chicks. The animals were perfused after a two day survival and sections from the brains were prepared by standard autoradiographic procedures. Adenosine derivatives were found to be transported in large amounts in both the anterograde and retrograde directions. Retrograde labeling of cell somata was found in the contralateral isthmo-optic nucleus after injection of the retina and in the oculomotor nucleus after injection of the extraocular muscles. Injections of the eye also led to heavy anterograde labeling of axons in the optic tract and in the tectum but little or no anterograde, transneuronal labeling of cell somata could be detected in the tectum.

Injections of 0.2 μl of 50 μCi/μl solutions of [^{3}H] adenosine made in the ventrobasal complex of the thalamus in rats lead to autoradiographic labeling of large numbers of cell somata in layer VI and of a smaller number of somata in layer V of the somatic sensory cortex. Little obvious cellular labeling is seen in the main zone of thalamocortical fiber termination (layer IV). Since this pattern of labeling is exactly the same as the retrograde cell labeling obtained after injections of horseradish peroxidase in the ventrobasal complex, it is tentatively concluded that the adenosine derivatives reach layers V and VI by retrograde axonal transport.

Anterograde, transneuronal transport of adenosine derivatives has been demonstrated by injections of [^{3}H] adenosine in the somatic sensory cortex of the rat. These result in labeling of cell somata in the thalamic reticular nucleus and in the pontine nuclei, cell groups that receive axons from but which do not project to the cortex. However, the additional cell labeling that occurs in these experiments in the thalamic ventral and intralaminar nuclear complexes and in the contralateral cerebral cortex may be the result of retrograde as well as anterograde transport of adenosine derivatives. (Supported by NIH Grants numbers NS 10526 and EY 00024.)

57 DISTRIBUTION OF ^{3}H RNA ALONG REGENERATING RABBIT HYPOGLOSSAL NERVE AFTER INJECTION OF ^{3}H URIDINE IN THE XII NUCLEUS. L. Autilio-Gambetti*, P. Gambetti. Div. Neuropath., U. of Pa., Phila., Pa.

Rabbits were injected with ^{3}H uridine at the level of the XII nucleus at different times after unilateral crush of the hypoglossal nerve. All animals were sacrificed 9 days after crush. At this time the hypoglossal nerve had regenerated 1.5-2 cm., as determined by ultrasturcture and by intraaxonal transport of ^{3}H glycoproteins. Both crushed and control nerves were dissected out, sectioned into 5 mm. segments, and the epineurium separated from each nerve segment. Nerves were washed with 0.2 N $HClO_4$ until no acid soluble radioactivity was present. RNA was extracted by hydrolysis in 0.3 KOH. No radioactivity was found in the epineurium, indicating that blood labeling was not significant. Both ^{3}H RNA and ^{3}H acid soluble fractions were increased in the regenerating nerve as compared to the control; an increase of 2-4 times in the radioactivity of the acid soluble fraction was observed only up to 2 days after injection. ^{3}H RNA was increased 6-9 times; one day after injection, this increase was detectable only in the nerve segments preceding the site of crush; 2-6 days after injection the increase was found in the regenerating segments, whereas the amount of ^{3}H RNA in the segments preceding the site of crush was similar to that of the control nerve. The amount of RNA (as determined by OD at 260 mμ in the RNA hydrolysate) was also found to be 3-5 times higher in the regenerating segments than in the control nerve. These results suggest that part of the RNA present in the regenerating hypoglossal nerve migrates from the hypoglossal nucleus. (Supported by NIH Grant NS-08933)

58 AXONAL TRANSPORT OF LOW MOLECULAR WEIGHT PROTEINS FROM THE ABDOMINAL GANGLION OF APLYSIA. R.W. Berry and A.W. Schwartz*. Dept. Anat., Northwestern Univ. Med. School, Chicago, Ill. 60611.

Low molecular weight proteins are synthesized in abundance by certain identified neurons of the abdominal ganglion of Aplysia and leave the cell body by a colchicine-sensitive process. The firm establishment of this process as axoplasmic transport and investigations of the further metabolism of these proteins have been hampered by the low efficiency of incorporation of precursors injected directly into these cells. We are therefore studying transport from the whole ganglion by isolating it in a chamber containing ^{3}H-leucine, while allowing the peripheral nerves to extend into an outer chamber containing excess unlabelled leucine. Following a 2 hr labelling period, acid-insoluble labelled material appears in the siphon, genital and pericardial nerves, migrating distally at about 3mm/hr at room temperature. This migration can be blocked by 1mM colchicine and will not pass a ligation. Separation on SDS-polyacrylamide gels indicates that a group of peptides at $\leq$ 9,000 daltons accounts for 30-40% of the leucine-labelled transported material. Labelled material of this molecular weight is not found in the connective nerves, nor is it synthesized by peripheral nerves incubated directly in ^{3}H-leucine, and is thus likely to represent the low molecular weight species transported from the identified cells. The proteolytic processing previously described in the somata of such cells continues in the axon as a time-dependent loss of 9,000 dalton material and concomitant appearance of peptides of 6,000, 3,000 and lower molecular weights.

Supported by USPHS grant NS 11519.

59 REVERSAL OF AXONAL TRANSPORT AT A NERVE CRUSH. Mark A. Bisby and Victor T. Bulger*, Division of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

We have investigated changes in retrograde axonal transport following a nerve crush, with the aim of identifying factors which might signal axonal damage. 3H-L-leucine was injected into the ventral horn of the lumbosacral spinal cord of both "intact" and "axotomized" rats. The latter group had a single crush made distally on their sciatic nerves at the time of injection or 6 hours later. At various intervals after injection all animals had double "collection" crushes made 14 mm proximal to the site of the initial distal crush. Labeled protein transported in orthograde and retrograde directions accumulated adjacent to the collection crushes over a 2-3 hour period, then the animals were killed and the nerves assayed. Labeled protein first reached the distal crush 5½ hours post-injection (p.i.) Significant retrograde transport to the collection crushes were detected in axotomized nerves during the 6-8 hour p.i. collection period, showing that there is a rapid reversal of transport at the distal crush, and that protein returning from the crush is transported at fast velocities (>130 mm/day). If the distal crush was made 6 hours p.i., the development of retrograde transport was delayed by one hour, indicating that an additional period of ½ hour was necessary for the crushed axon to develop the ability to reverse transport. Over the period 6-20 hours p.i. retrograde transport was 55% higher in axotomized nerves than in intact nerves. Peak retrograde transport occurred 9-11 hours p.i. in axotomized nerves and 44-47 hours p.i. in normal nerves. Changes in the magnitude or time-course of retrograde transport following injury to motoneuron axons may be signals which initiate chromatolytic changes in the cell body.

60 RNA DISTRIBUTION IN GOLDFISH OPTIC TECTUM AFTER INTRACRANIAL INJECTION OF ^{3}H-URIDINE. AN EM AUTORADIOGRAPHIC STUDY DURING OPTIC NERVE REGENERATION. P. Gambetti, L. Autilio-Gambett*, N. Ingoglia, P. Weis*. Div. Neuropath., U. of Pa., Phila., Pa. and Depts. Physiol. and Anat., New Jersey Med. School, Newark, N.J.

In a previous study, we have analyzed by EM autoradiography the distribution of ^{3}H RNA present in the goldfish optic tectum following intraocular injection of ^{3}H-uridine during optic fiber regeneration. This study has shown that growth cones and adjacent glial cells have the highest density of grains related to ^{3}H RNA whereas other structures are much less labeled. This study, however, has not established whether the ^{3}H RNA present in the regenerating axons is transported from the ganglion cells of the retina or whether it is synthesized in the tectum by the glial cells and then transferred into the axon. To answer this question the distribution of ^{3}H RNA present in the goldfish tectum following intracranial injection of ^{3}H-uridine has been analyzed by EM autoradiography. Goldfish were sacrificed 24 days after crush of both optic nerves. Six days prior to sacrifice, ^{3}H-uridine was injected intracranially over the tecta. Preliminary data show that glial as well as vascular cells have a density of grains related to ^{3}H RNA 10 - 20 times higher than any other structure; whereas, the grain density over regenerating axons and growth cones is almost negligible. These findings suggest (a) that during axonal regeneration there is no significant transfer of ^{3}H RNA from glial cells to regenerating axons, and (b) that the ^{3}H RNA present inside the regenerating axons and growth cones following intraocular injection of ^{3}H-uridine is not synthesized in the tectum but migrates from the retina.

(Supported by NIH Grants NS-08933 and NS-11259.)

61 KINETICS OF AXONAL TRANSPORT OF SEROTONIN IN A SINGLE AXON. Daniel J. Goldberg, Ariel A. Sherbany* and James H. Schwartz. Div. Neurobiol. & Behav., Dept. Physiol., Columbia U., Coll. Phys. & Surg., N.Y. 10032

Previous studies of axonal transport have used nerves containing many axons. In order to elucidate the kinetics of transport in a single axon, we have been studying the transport of ^{3}H-serotonin (^{3}H-5HT) in the giant cerebral neuron (GCN), a serotonergic cell of *Aplysia californica*. We have postulated a model of axonal transport involving a concentration-dependent, intermittent binding of organelles to the translocation machinery in the axon (Goldberg, Goldman and Schwartz, J. Physiol., in press). The present experiments were designed to test some predictions of this model.

A pulse of ^{3}H-5HT in the lip nerve axon of the GCN was obtained by injecting ^{3}H-5HT and then, 70 min later, ligating the lip nerve where it exits from the cerebral ganglion. Nerves were maintained in sea water supplemented with nutrients at either 23° C or 14° C for varying periods of time, and then sequentially sectioned to yield transport profiles. ^{3}H-5HT was transported as a discrete peak. This kind of experiment can provide two independent kinetic parameters: the *speed* at which a peak is translocated, and the *peak width*. We found that ^{3}H-5HT was translocated with rates of about 120-140 mm/day at 23° C and about half that speed at 14° C. We are as yet uncertain whether these rates remain constant with time. We have consistently observed that the peak widens as it travels along the axon, oftentimes becoming skewed in the proximal direction. This spread in the distribution of transported material is predicted by our model for translocation.

62 EARLY CHANGES IN THE METABOLISM OF GOLDFISH RETINAL GANGLION CELLS FOLLOWING AXOTOMY. Bernice Grafstein and Roberta Alpert*. Physiol. Dept., Cornell Univ. Med. Coll., New York, N.Y. 10021.

Autoradiographic studies have previously shown that at 4-7 days after the optic axons in goldfish have been cut there is an increase in the incorporation of amino acid into protein in the cell bodies of the retinal ganglion cells (1). In the present study, changes were detected as early as 18-24 hours after axotomy by measuring the amount of labeled material appearing in the optic nerve 6 hours after the injection of ^{3}H- or ^{14}C-proline into the eye. Labeled protein in the nerve (presumably conveyed by axonal transport) showed an increase of nearly 60% by 24 hours after optic tract section, while the labeled trichloracetic acid-soluble fraction (mostly free amino acid) was only increased by about 20%. The increase in protein was significantly greater ($p < .02$) than the increase in free amino acid, even at 24 hours. Between 1 and 5 days after axotomy the amount of labeled protein in the nerve nearly doubled, while the amount of labeled acid-soluble material remained at its 1-day level. Phospholipids labeled with ^{3}H-glycerol and nucleotides labeled with ^{3}H-guanosine showed little or no increase until 5 days. It is therefore possible to distinguish two metabolic changes in the axotomized cells, one occurring within 24 hours after axotomy, the other when axonal outgrowth begins.

(Supported by USPHS Grant NS-09015 from NINCDS).

(1) Murray, M. & Grafstein, B. Exptl. Neurol. 23, 544 (1969).

63 CALCIUM BINDING PROTEIN IN BRAIN SYNAPTOSOMES. Z. Iqbal and S. Ochs. Dept. Physiol., Indiana Univ. Medical Center, Indianapolis, In. 46202.

We have recently reported that ^{45}Ca is carried down in peripheral nerve at the usual fast transport rate of 410 mm/day bound to a 15,000 MW protein (Iqbal, Z. and Ochs, S., Neurosci. Abs. 1:802, 1975). This communication describes a similar Ca binding protein (CaBP) of 15,000 molecular weight in the synaptosomes isolated from cat brain. The synaptosomes prepared according to the method described by Gray and Whittaker (J. Anat. 26:79, 1962) were incubated with ^{45}Ca in 0.32M sucrose medium containing 10 mM Tris-HCl, pH 7.5, 1.0 mM mercaptoethanol, 5 mM ATP, 3 mM Mg and 0.1 mM Ca at 38° for 10 minutes with constant shaking. They were washed thoroughly in cold sucrose solution and subjected to osmotic shock. The soluble fraction obtained after high speed centrifugation (105,000 g for 1 h) was lyophilized and chromatographed through Sephadex G-100 and Biogel A 5m columns using 10 mM Tris-HCl buffer, pH 7.5 containing 0.1 mM mercaptoethanol and 0.1 mM Ca. In another series of experiments the incubation with ^{45}Ca was performed with the soluble fraction obtained after osmotic shock of the synaptosomes. Free ^{45}Ca was removed by filtering through Amicon Diaflow membrane UM-2 and the samples chromatographed on the columns. The ^{45}Ca activity in these preparations falls at the same position in the column eluates as does the 15,000 MW CaBP isolated from nerve. After several steps of purification including Diaflow membrane filtration, ammonium sulfate fractionation, gel filtration and acrylamide electrophoresis, the protein was tested for its ^{45}Ca binding capacity by equilibrium dialysis technique at 25°C and the apparent dissociation constant appears to be 6.66 x 10^{-5}M. We consider it likely that the CaBP is transported into the synapse to play a functional role at that site. Supported by PHS ROI NS 8706-07 and NSF Grant BNS 75 03868-A02.

64 METABOLIC ALTERATIONS INDUCED IN "FAST" AND "SLOW" SKELETAL MUSCLES OF RAT BY SILASTIC CUFFS PLACED ON MOTOR NERVES. F.C. Kauffman and E.X. Albuquerque. Dept. Pharmacol. Expt. Ther., Sch. of Med., Univ. of Maryland, Baltimore, MD. 21201.

Previous studies (Kauffman et al., Exp. Neurol. 50:60, 1976) showed that chronic application of cuffs containing vinblastine to the sciatic nerves of rats induced marked alterations in glucose-6-P and phosphocreatine (PCr) in extensor digitorum longus (extensor) muscles. Since drugs readily diffuse from silastic cuffs and appear in various tissues, we examined concentrations of metabolites in "fast" extensor and "slow" soleus muscles ipsilateral and contralateral to cuffed nerves. One group of rats had silastic cuffs without drug applied to the sciatic nerve and a 2nd and 3rd group were exposed to cuffs containing 0.1% vinblastine and 0.1% colchicine, respectively. A significant decrease in PCr levels occurred in extensor muscles supplied by nerves cuffed without drug; therefore, changes in this metabolite in extensor muscles may occur in the absence of disruption of axoplasmic flow by the silastic cuff technique. Electrical stimulation of nerves exposed to cuffs containing colchicine or vinblastine elicited muscle twitiches; however, extrajunctional acetylcholine sensitivity was increased to about 160-250 mV/nC (a value 3-4 fold lower than 10-day chronically denervated muscles). Levels of glucose-6-P, lactate, alpha-ketoglutarate, PCr and ATP in contralateral muscles were the same in each of the three groups. Thus, alterations in metabolites in muscles innervated by cuffed nerves cannot be explained by a direct action of vinblastine or colchicine on muscle metabolism. (Supported in part by USPHS grant NS-12063, the Paralyzed Veterans of America and the MDA.)

65 TAURINE IS AXONALLY TRANSPORTED IN THE GOLDFISH VISUAL SYSTEM.
T.D. Lindquist*, N.A. Ingoglia, J.A. Sturman and G.E. Gaull. N.J. Med. Sch., Newark, N.J. 07103 and Instit. Res. Ment. Retdn., Staten Island, N.Y. 10314.

Taurine is present in high concentrations in nervous tissue. Recent evidence has suggested that taurine may function as a neurotransmitter or a neuromodulator in brain as well as in retina. The present experiments were conducted in order to see if taurine is axonally transported in the goldfish visual system.

^{35}S-taurine was injected into the right eye of a group of fish. At various times after injection, fish were sacrificed and their retinas and tecta were assayed for radioactivity. Large differences in radioactivity in left vs right tecta (indicating axonal transport) were apparent as early as 1 day after injection and were still evident 21 days later. Analysis of tectal radioactivity by an amino acid analyzer linked to a flow cell scintillation spectrometer showed all the radioactivity to be present as taurine. When ^{35}S-cysteine, a precursor of taurine, was injected in the same system, radioactive material was also found to be axonally transported. Analysis of tectal radioactivity, however, showed the presence of only ^{35}S-taurine, inorganic sulfate and proteins, with no evidence of ^{35}S-cysteine. Injections of ^{14}C-GABA, an amino acid homologue of taurine, as well as a putative neurotransmitter, resulted in little or no transport of radioactivity. These results demonstrate a rapid and relatively specific axonal transport of taurine in the goldfish visual system.

66 ULTRASTRUCTURAL STUDIES OF THE DEVELOPING IRIS MUSCULATURE IN THE CHICK EMBRYO WITH SPECIAL REFERENCE TO NEURONAL DEATH IN THE CILIARY GANGLION.
Y. Narayanan* and C. H. Narayanan. Dept. of Anat., L.S.U. Medical Center, New Orleans, Louisiana, 70119.

The purpose of the present investigation was to examine at the ultrastructural level the differentiation of the iris musculature of the chick embryo during development in an attempt to study possible interactions between outgrowing nerve fibers from the ciliary ganglion and their target iris muscle cells. Iris muscle was dissected out from chick embryos between days 9 and 13 of incubation and processed for electron microscopy. This time period was chosen to coincide with the time course of cell loss in the ciliary ganglion of the chick. The muscle cells of the iris between days 9 to 12 of incubation consisted mainly of undifferentiated myoblasts or myotubes. Myofibrils were not observed until day 13. The cells showed abundant hypertrophied golgi from which large coated vesicles were found to arise by budding. These golgi derived vesicles were found to migrate to the cell surface forming omega figures and become incorporated in the cell membrane, suggesting the release of a trophic substance essential for the establishment of a successful relationship between the outgrowing nerve fiber and the target cell. On the basis of these observations, we postulate: (a) a transfer of material of some sort from the target cell to the neuron in retrograde fashion which ensures the survival of the neuron; and (b) a transfer of material from the neuron to the target cell in anterograde fashion which triggers the differentiation of the target cell.

This was supported by USPHS grant #R01 DE04258-01.

67 ISOLATION AND PARTIAL PURIFICATION OF TRANSNEURONALLY TRANSPORTED MATERIAL IN THE VISUAL SYSTEM. S. Reinis*, J.M.Goldman*, J. Kleib*, and P. Black*. Dept. Psych., U. of Waterloo, Waterloo, Ont., Canada.

In 1975, we reported that after an intraocular injection of 3H-proline the radioactivity may be found around and most probably within the main dendrites of cortical neurons (Trans. Am. Soc. Neurochem. 6: 108, 1975). At least 45% of this radioactivity could be extracted from the tissue with distilled water or several 0.01M buffers at p.H 7.0 - 7.2. Of the remaining radioactivity, 95% was solubilized with SDS. Disc electrophoresis with subsequent liquid scintillation counting or autoradiography showed that the labeled material migrates, relatively slowly, in one single band, as did the SDS - extracted radioactivity. Ammonium sulphate precipitation revealed one peak of radioactivity at 30% saturation and another at 50% saturation. The second peak is, however, much lower when the proteinases of the extracts are inhibited. DEAE - Sephadex chromatography of the 30% peak yielded a single radioactive wave eluted at p H 6.6. The molecular weight of this material has been estimated by SDS electrophoresis.

68 BIOSYNTHESIS AND AXONAL TRANSPORT OF MEMBRANE GLYCOLIPIDS IN R2, A SINGLE IDENTIFIED NEURON OF *APLYSIA CALIFORNICA*. Ariel A. Sherbany*, Richard T. Ambron, and James H. Schwartz. Div. of Neurobiol. & Behav., Dept. Physiol., Columbia U., Coll. Phys. & Surg., N.Y. 10032.

Previous work by others on axonal transport of lipids is complicated by the probable local incorporation of precursors in axons and glial cells; in addition, incorporation of most lipid precursors is relatively slow, presumably because most classes of lipids do not turn over rapidly. We have introduced ^{3}H-N-acetylgalactosamine (^{3}H-NAGA) directly into the cell body or axon of R2 by injection, and therefore have been able to study transport of rapidly-labeled glycolipids. 15 hours after *intrasomatic* injection, ^{3}H-glycolipids appeared in the cell body and also far along the right connective, which contains the major axon of R2. To show that the ^{3}H-glycolipids formed after *intrasomatic* injection were within R2, we dissected out the cell body and also extruded axoplasm. We characterized by polysilicic acid glass fiber chromatography 4 *membrane* glycolipid components which are synthesized in R2's cell body. These could not have been formed in glial cells, since the ^{3}H-glycolipids synthesized by non-neuronal tissue components chromatographed quite differently. Rather compelling evidence that ^{3}H-glycolipids were actually transported from the cell body, and were not produced locally after diffusion of precursors, was obtained by direct *intraaxonal* injection of ^{3}H-NAGA: we found that only one lipid component was labeled in the axon. This lipid differed from those synthesized in the cell body, and exported into the axon. We are now characterizing the individual glycolipids and investigating their distribution along the axon to determine the selectivity and rates of their axonal transport.

69 A GENETICALLY POLYMORPHIC POLYPEPTIDE IS TRANSPORTED DOWN THE AXONS OF RABBIT RETINAL GANGLION CELLS, AS PART OF AN ADDITIONAL (FIFTH) GROUP OF TRANSPORTED PROTEINS. Mark B. Willard* and Karen L. Hulebak*. (SPON: T. A. Woolsey) Dept. Anat. & Neurobiol. and Biochem., Washington Univ., Sch. Med., St. Louis, Mo. 63110.

A polypeptide (H) in the rabbit nervous system is genetically polymorphic; one of its forms (H^1, MW = 200,000 daltons) has an electrophoretic mobility that is 6% less than a second form (H^2). In order to determine whether the H polypeptide is a neuronal, intra-axonally transported polypeptide, ^{35}S-methionine was injected into the eyes of rabbits, and the temporal sequence of arrival of labeled transported polypeptides in segments of the retinal ganglion cell axons was analyzed by SDS gel electrophoresis followed by autoradiography of the gels. Label associated with the H polypeptide was identified as an autoradiographic band which had a different electrophoretic mobility in strain X/J (an inbred rabbit strain which has only the H^2 form of the polypeptide) than in an outbred strain (in which H^1 is the predominant form). The H polypeptide became labeled in a sequence suggesting that it is transported at a velocity of about 1 mm per day.

We previously described four groups of polypeptides transported in the rabbit retinal ganglion cells at velocities of 250, 34-68, 4-8, and 2-4 mm per day. The H polypeptide and two coordinately labeled polypeptides make up a fifth group with a slower velocity than any of the groups previously described. The composition of this group (three intensely labeled polypeptides, one with a molecular weight of about 200,000 daltons) is similar to the composition of a slow phase of transport described in the rat ventral motorneurons and the cat spinal sensory neurons (Hoffman and Lasek (1975) J. Cell. Biol., 66: 351). (Supported by NINCDS NS 12450 and a Neuromuscular Research Center Grant.)

70 THE EFFECT OF REPETITIVE ELECTRICAL STIMULATION ON AXOPLASMIC TRANSPORT. R. M. Worth* and S. Ochs. Depts of Physiol., Neurol. Surg. and the Med. Biophysics Program, Indiana Univ. Medical Center, Indianapolis, In. 46202.

A relationship of increased levels of neuronal activity to the axoplasmic transport of materials was studied in nerve fibers in Vitro using high rates of repetitive electrical stimulation. Other investigators had found no change in the transport rate, but previous experiments from our laboratory appeared to demonstrate a 10% reduction in rate at 100 pps (Ochs, S. and Smith, C., Fed. Proc., 30:665, 1971; Ochs, S. and Worth, R., Science, 187:1087, 1975). Subsequently, temperature inequalities were discovered in the chamber system used and it appeared that a temperature differential might account for the results earlier obtained. The present study, therefore, was undertaken to clarify this point using two temperature controlled chambers. For measurement of downflow, the L7 dorsal root ganglia of 11 cats were injected as usual with ^3H-leucine and 2 hours later, the sciatic nerves were removed and placed in chambers, one for repetitive stimulation, the other as a control. The duration of stimulation varied from 1-3 hours but the frequency of stimulation was kept constant at close to 350 pps and action potentials monitored. At the conclusion of the stimulation period, the nerves were removed and prepared as usual to show the distribution of activity of labeled materials and the rate of axoplasmic transport assessed from the position of the foot of the crests. These experiments reveal an apparent reduced rate of axoplasmic transport in response to electrical stimulation. The control rate at 38°C was 432 ± 14 (S.E.) mm/da. The rate for the stimulated nerves was 374 ± 10 (S.E.) mm/da. The 15% reduction is significant at the $P < .01$ level. Work is now in progress to determine if the effect of the high level of stimulation is to cause a temporary arrest as previously noted, or a reduction in rate throughout the whole period of stimulation. Supported by PHS ROI NS 8706-07 and NSF Grant BNS 75 03868-A02.

Basal Ganglia

71 Postnatal Synaptogenesis in the Caudate Nucleus. A.M. Adinolfi, Dept. of Anatomy, Sch. of Med., Mental Retardation Research Ctr., UCLA, Los Angeles 90024.

This study used light and electron microscopy to describe changes in the synaptic organization of developing caudate nucleus and to quantitate postnatal synaptogenesis in this region. The caudate nuclei from 45 kittens, ranging in age from newborn to 49 postnatal days, were processed for histology (20 kittens, Rapid Golgi and Golgi-Cox methods) and for electron microscopy (25 kittens). Observations at the light microscopic level focused on the perinatal period and suggest an early maturation of spiny interneurons of the caudate nucleus. Golgi impregnation of these cells at early postnatal ages (birth to 5 days) revealed 3 to 5 primary dendrites which radiate from the cell body and extend for distances of 10 to 20 um before branching. Secondary dendritic branches contain spines and extend, with further branching, for additional distances of 30 to 80 um. The dendritic fields of neighboring caudate neurons overlap and the axons which arise from these cells course and branch within the dendritic fields.

Examination of perinatal caudate neuropil (birth to 5 days) by electron microscopy revealed an extensive and well-developed axodendritic connectivity. Axonal profiles form multiple synapses en passant along single dendrites and dendritic spines or on several adjacent dendritic branches. At these ages, however, few of the terminals are filled with synaptic vesicles and mitochondria and few synaptic contacts are distinctly asymmetrical. Instead, axon terminals with scattered vesicles form synaptic contacts which are often symmetrical or slightly asymmetrical. By the eighth to tenth postnatal days, most boutons are filled with vesicles, most junctional complexes are distinctly asymmetrical, and axodendritic connectivity has been modified further by the increase of postsynaptic dendritic spines and branchlets. Axosomatic connectivity and desmosomal junctions between adjacent dendrites are additional features of the immature caudate neuropil.

Quantitative estimates of postnatal synaptogenesis were made from electron micrographs by determining the number of synapses/ 100 um^2 of caudate neuropil. The results obtained from counting 5124 synapses in 25 kittens of varying ages showed the following relationship between age and synaptic density:

Age (days)	Mean No. Synapses/ 100 um^2 Tissue
0-2	3.5
4-6	6.7
8-10	7.5
12-15	8.5
18-23	12.4
28-49	13.2

These results suggest that 1) the basic pattern of synaptic organization of the caudate nucleus is established within the first postnatal week, and 2) subsequent changes in synaptic organization are primarily quantitative. The increase in synaptic density during postnatal development parallels the development of synaptic security with age in the kitten as shown by our electrophysiological studies of corticostriatal (Lidsky, et al., Exp. Neurol., 50: 283, 1976), thalamostriatal (Fuller et al., this session) and nigrostriatal (Morris et al., this session) projections which report an increase in the number of units responding to, and following repetitive stimulation of, these afferent fibers during first two months post-partum.

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72 Kitten Caudate Nucleus Development I. **Thalamo-Caudate Connections.**
D.R.G. Fuller,* C.D. Hull and R. Morris.* **Mental Retardation** Research Ctr. Brain Research Inst., UCLA, Los Angeles, 90024.

The development of thalamo-caudate connections was studied in kittens ranging in age from one to fifty days postnatally. Extracellular single units were recorded from ipsilateral caudate nucleus in response to stimulation of the thalamus and precruciate cortex. Anatomical studies (see Adinolfi - Postnatal Synaptogenesis in the Caudate Nucleus, this meeting) show that synapses are present in caudate nucleus at birth. The density of these synapses increases with age. Previous studies of cortical-caudate projections show that this increase in synaptogenesis is accompanied by electrophysiological changes in that the number of neurons driven by cortical stimulation increases with age and the latencies of cortically evoked responses decrease with age; confirmation of these effects has been obtained (e.g. 50 msec., day 3; 33 msec., day 20; 28 msec., day 40) in this series of experiments.

Similarly, thalamically evoked responses in caudate nucleus also decrease in latency with age (e.g. 35 msec. at day 10, 22 msec., day 30). Although some cells could not be fired by either thalamic or cortical stimulation, the proportion of these decreased with age. Other cells were evoked by one or both of the stimulated sites. Of the cells which were evoked by stimulation of either site the majority (70%) had shorter response latencies to thalamic than to cortical stimulation. This finding suggests that a direct thalamic-caudate pathway, rather than a thalamic-cortico-caudate pathway has been utilized. Further evidence of a direct thalamic-caudate pathway was obtained from the small variation in response latencies (S.D. $\leq$ 1.0 msec.) in some neurons. In general, the ability to follow repetitive stimulation increases with age, although some exceptions to this statement were found. It is quite often easier to evoke responses at 3 hz than at 1 hz and sometimes in order to evoke a reliable response, 10 hz stimulation needed to be used before 3 hz would evoke a response. This fact is probably not indicative of a lack of transmitter, but rather that long lasting cumulative depolarization finally resulted in a threshold response.

A small number of interactions have been studied in kittens over 20 days. These indicate that cortical stimulation besides evoking a response in caudate cells can inhibit thalamically evoked responses, a situation similar to that found in the adult preparation. This study indicates that a functional thalamo-caudate pathway is present in neonatal kittens as early as the first five days of age. With increasing age the response latencies decrease, and the numbers of responsive units and their ability to follow repetitive stimuli increases.

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73 PERMANENT DEFICITS IN CAUDATE NEURONAL ACTIVITY INDUCED BY NEONATAL CORTICAL DAMAGE. E. Garcia-Rill, N.A. Buchwald, C.D. Hull, M.S. Levine, J. Villablanca. Mental Retardation Research Ctr., Brain Research Inst., UCLA, Los Angeles, 90024.

The fact that axons of cerebral cortical neurons project to the caudate nucleus and putamen has been well established from the morphological viewpoint. Convincing neurophysiological data now exists to indicate that this corticofugal output is excitatory upon striatal neurons. This conclusion is a result of two kinds of physiological data; the first is the fact that the initial response evoked by cortical stimulation and recorded intracellularly in caudate neurons is usually an EPSP, and the second is that the spontaneous firing of caudate nucleus neurons slows markedly in cats which have sustained frontal cortical damage. A major interest of our laboratory is the elucidation of possible compensatory mechanisms after early brain injury. For example, we have been studying the behavioral, neurophysiological and electrophysiological development of cats subjected to frontal cortical lesions during the first month of life, and comparing these animals with their littermate controls. The experiment mentioned above, in which frontal cortical lesions resulted in slowing of caudate neuronal activity, was performed entirely on adult cats. It seemed important to ask whether the excitatory cortical control of the striatum was present in infancy as well as in the adult, and if it was, whether compensatory mechanisms exist to enable the caudate nucleus to "recover" from interruption of this excitatory control produced in infancy. In an attempt to answer these questions partially, bilateral lesions of the frontal cerebral cortex were carried out on a series of kittens ranging from 9-36 days of age. This paper will report on the spontaneous activity of caudate neurons in these animals which were allowed to survive to juvenile-young adult ages (9-18 months). Similar studies of animals, frontally decorticated in infancy and allowed to reach full maturity before measures of neuronal activity are made, have not yet been completed.

In a terminal experiment, the spontaneous firings of pairs of caudate neurons were sampled simultaneously from both caudate nuclei as previously described (Hull et al. Brain Res. 73, 1974). The mean interspike interval (ISI) for caudate cells of neonatally-lesioned juvenile cats was 5280 msec compared to 3175 msec for caudate cells of non-lesioned controls. In cats in which the frontal cortex was ablated in adulthood the mean ISI was 4854 msec compared to a mean ISI of 1712 msec for caudate neurons of their controls. An analysis of variance indicated that the differences between non-lesioned and lesioned animals in both adult and juvenile conditions was statistically significant ($p \lesssim .01$). Of particular interest was the finding that the non-lesioned juvenile cats displayed slower spontaneous caudate unit firing rates than non-lesioned adult cats. This datum as well as other evidence leads us to believe that the mature level of spontaneous firing in the striatum is a slowly developing phenomenon. We know from previous research (Lidsky et al. Exp. Neurol. 50, 1976; Adinolfi, Soc. for Neurosci. 5, 1975) that functional excitatory cortico-striate connections exist in neonates. These data together with the results presented in the present paper show that compensation for the tonically induced cortical excitation does not occur, at least for the first year of postnatal life.

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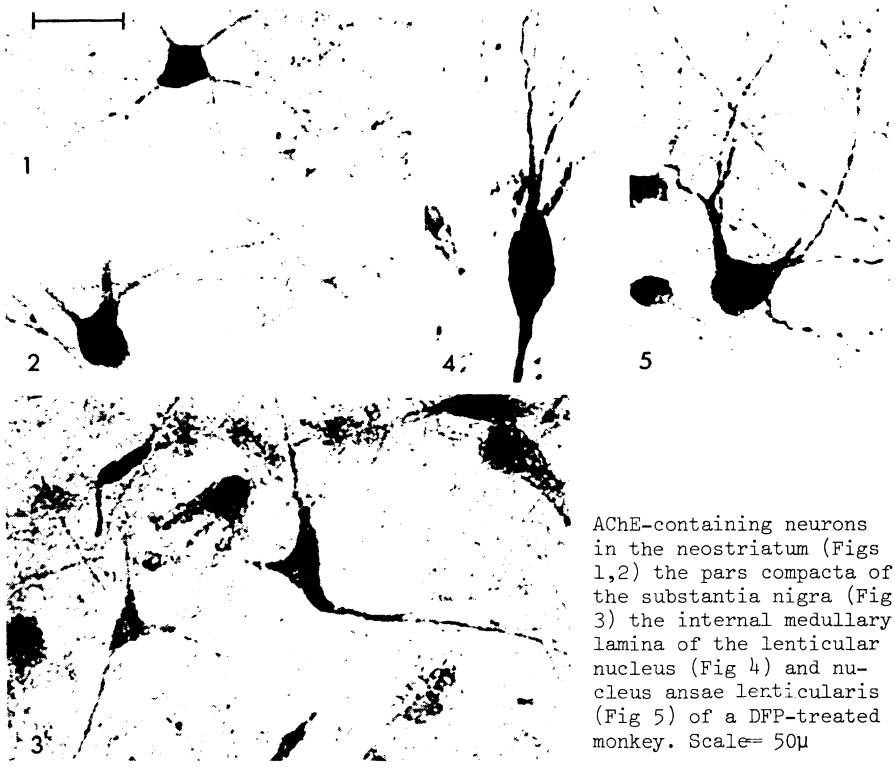
74 EPITHALAMIC AND PONTINE PROJECTIONS OF THE FELINE ENTOPEDUNCULAR NUCLEUS. Kenneth D. Larsen* and Russell L. McBride* (SPON: Jerome Sutin). Depts. Physiol. and Anat., Emory Univ., Atlanta, GA., 30322.

The entopeduncular nucleus (EPN) is the feline homologue of the medial segment of the globus pallidus and a major source of efferents from the basal ganglia, projecting to the thalamus, epithalamus and pons. Projections to the thalamus are known to influence the cerebello-thalamo-cortical relay, thereby affecting motor activity, while the functional significance of the epithalamic and pontine projections is unknown. There is evidence for topographic organization of cortico-caudate and caudato-pallidal projections, but the organization in cats of EPN projections has not been shown. To determine if there is topographic localization within EPN of efferents to epithalamus and pons, the EPN cells have been studied by antidromic activation and retrograde transport of horseradish peroxidase. Extracellular recordings were made from EPN neurons antidromically activated by stimulation of the lateral habenular and pedunculopontine nuclei. In other experiments, these same areas were injected with horseradish peroxidase and the numbers and location of labeled cells in EPN determined. Both techniques revealed that EPN cells projecting to the lateral habenular and pedunculopontine nuclei originate from all portions of the nucleus. Of 201 sampled neurons, 13 cells were antidromically activated by lateral habenular stimulation; latencies ranged from 1 to 3 msec with a mode of 2 msec (conduction velocity of less than 5 m/sec). EPN cells projecting to pedunculopontine nucleus (16 of 201 neurons) have 1 to 2 msec latencies to antidromic activation (conduction velocity of less than 12 m/sec). Using a standard systematic sampling technique, EPN was estimated to contain 3800 neurons. In typical peroxidase experiments, 16% of EPN cells were labeled after lateral habenular injections, and 1% after pedunculopontine injections. While neurons were distributed throughout EPN following lateral habenular injections, the more compact central zone of the nucleus contained proportionally fewer labeled neurons than more dorsal and medial zones where cells are intermingled among the fibers of the internal capsule and adjacent lateral hypothalamus. (Aided by NIH Grant NS-06948.)

75 LOCALIZATION OF ACETYLCHOLINESTERASE WITHIN EXTRAPYRAMIDAL AND RELATED STRUCTURES IN THE BRAINS OF MONKEYS TREATED WITH DFP. L.J. Poirier, R. Marchand* and A. Parent. Neurobiology Lab. and Dept. Anat., Laval Univ. Quebec, QUE. G1K 7P4.

The pharmaco-histochemical technique based on the administration of diisopropylfluorophosphate (DFP) and on an histochemical technique for cholinesterases, as applied by Butcher et al., J. Neural Transm. 37: 127, 1975) in the rat, was used to study the distribution of acetylcholinesterase (AChE) within the extrapyramidal system. Under such conditions large neurons (possibly two types) located within the caudate nucleus and the putamen show an intense AChE activity. Somewhat larger neurons which also display an intense activity are present within the external and internal medullary laminae of the lenticular nucleus, at the base of the anterior limb of the internal capsule, and within nucleus ansae lenticularis, or else, they are interspersed between neurons of the nucleus of the diagonal band of Broca and the substantia innominata (nucleus basalis of Meynert). Medium-sized neurons of the external and internal divisions of the pallidum and of the pars diffusa of the substantia nigra show a moderate AChE activity. Elongated neurons with an intense AChE activity and ovoid neurons with a moderate AChE activity are present in the pars compacta whereas the few and somewhat larger neurons of the pars lateralis of the substantia nigra have a moderate AChE activity. An intense AChE activity may be observed in the soma and processes of the neurons of nucleus tegmentosus pedunculopontinus, subnucleus compactus, and in the soma of the neurons of the locus coeruleus whereas the soma of the neurons of the subthalamic nucleus and nucleus ansae peduncularis show a weak to moderate AChE activity. (Supported by the Medical Research Council of Canada).



AChE-containing neurons in the neostriatum (Figs 1,2) the pars compacta of the substantia nigra (Fig 3) the internal medullary lamina of the lenticular nucleus (Fig 4) and nucleus ansae lenticularis (Fig 5) of a DFP-treated monkey. Scale= 50µ

76 THE EFFECTS OF ROSTRAL CAUDATE NUCLEUS LESIONS ON MOTOR BEHAVIOR IN THE CAT. G. T. Sanders*, M. E. McNeill, M. L. Murphey*, D. L. Bragunier*, and M. C. Metz*. East Carolina Univ., Greenville, NC. 27834.

Facilitatory as well as inhibitory areas have been described in the rostral caudate nucleus (CN) of the cat (Liles and Davis, 1969, Science, 164). Lesions involving the inhibitory (anteroventral) area, sparing the facilitatory (dorsoposterior) area, have been reported to result in athetoid and choreiform movements in a limited number of cats (Liles and Davis, 1969; Harik and Morris, 1973, Brain Res., 62).

To further investigate the behavioral heterogeneity of the rostral CN, this study was undertaken to (1) investigate the behavioral effects of unilateral, stereotaxic electrolytic lesions in the rostral CN of the unrestrained cat, (2) objectively quantify hyperkinesia (height of forepaw rise and abduction during ambulation), and (3) design and build a model for the measurement of the amplitude of hyperkinesia. Lesions in 8 adult cats were verified in frontal sections stained with the Klüver-Barrera method. Unrestrained motor observations were made 15 min/day x 10 days pre and post operatively. An incremental glass runway was used for measurements during ambulation.

The primary postoperative motor changes were bilateral forelimb chorea which was observed in 3 cats, 2 of which also displayed ipsilateral hindlimb chorea and 2 of which displayed athetosis. All of these lesions were between coordinates A 19.5 and A 17.0 (Snider and Niemer, 1961). Other behaviors that changed in frequency included head shaking, sidelying, self and other cat grooming, sphinx position, crouching, rubbing, kneading and mewing. An incidental finding was bilateral circling (predominantly tail chasing) in 2 cats with lesions rostral to the CN and in 1 cat with a lesion involving the anteroventral CN. The incremental glass runway was economically and functionally useful for the measurement of height of forepaw rise and abduction during ambulation. In this study the varied findings of bilateral forepaw chorea, ipsilateral hindlimb chorea and bilateral circling following small (1-2 mm) unilateral lesions not only support the hypothesis that there is functional localization in the caudate but strongly suggest that it is more specific than previously reported.

77 TONIC FIRING PATTERNS OF SUBSTANTIA NIGRA NEURONS IN AWAKE MONKEYS, Marjorie E. Anderson. Depts. of Rehabilitation Medicine and Physiology and Biophysics and Regional Primate Research Center, Univ. of Wash., Seattle, Wa. 98195

Tonic firing patterns of neurons in the substantia nigra of monkeys have been studied during active maintenance of a stable postural position, in an attempt to establish usable criteria that would distinguish between thalamic-projecting neurons of the pars reticulata and nigrostriatal neurons during motor performance tasks. Juvenile monkeys (M.mulatta) were trained to maintain a stable head position in which a skull-mounted mirror would reflect a light beam from an overhead source into a fixed position photodetector cell. Following training, a recording chamber was surgically implanted, and after recovery and retraining, the firing patterns of neurons in the substantia nigra (and subthalamic nucleus) were recorded with a stable chair position. Interspike interval histograms of this tonic activity were compared with those reported previously for neurons in the other basal ganglia output nucleus, the internal globus pallidus(GPi). Like GPi neurons, cells in the pars reticulata have high frequency, rather regular discharge patterns, most with mean frequencies higher than 50/sec. Neurons on the dorsal border and more medial portions of the substantia nigra have lower frequency, more irregular discharge patterns. Attempts are under way to determine, by antidromic activation from chronically implanted electrodes, whether these tonic firing patterns can be used to distinguish nigrothalamic (or nigrocollicular) from nigrostriatal neurons. (Supported by NIH grants NS10804 and RR 00166 and SRS grant16-P-56818)

78 SOMATIC AND VISUAL FEEDBACK TO MONKEY CAUDATE NUCLEUS DURING A CENTRAL MOTOR PROGRAM. R.J. Anderson, J. Wayne Aldridge*, and J.T. Murphy. Dept. of Physiol., Univ. of Toronto, Toronto, Ont., Canada.

Experiments were designed to test whether either somatic or visual sensory information is channelled to caudate nucleus during voluntary movement. Monkeys (M. speciosa) were trained to track a visual target by means of flexion-extension movement about the wrist. Target and manipulandum positions were presented on the visual display for the monkey. Torques were randomly delivered to the manipulandum thus activating cutaneous and deep receptors in the hand and limb. Somatic and visual feedback were randomly decoupled during the motor task, thus providing indication of the relative role of each sensory channel.

The activities of 60 single neurons in contralateral caudate nucleus were recorded both with the monkey at rest and during performance of the motor task. Somatic sensory stimulation with the animal in the resting state produced a response in 36 of these neurons. The neurons typically had wide receptive fields, often involving more than one limb. Cells usually responded to stimulation of either deep receptors in muscles or joints, or of cutaneous receptors; a small percentage (8 of 36 cells) responded to both types of passive stimulation.

One-half of the cells studied responded during some component of the motor task. A convergence of visual and somatic feedback was demonstrated in this context. Of 30 task-related cells, 16 responded only to somatic feedback during task performance, while 14 responded to both somatic and visual feedback. No task related cells were linked solely to visual feedback. These findings indicate that both somatic and visual information play an important role as determinants of output from caudate neurons during voluntary movement, with the somatic feedback channel predominant.

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79 IS NIGRONEOSTRIATAL FACILITATION DOPAMINERGIC OR NOT ? Peter Brawley, James Doyle* and Charles Marmar*. Dept. Psychiat. Res. Div., Toronto General Hospital, Toronto M5G 1L7, Canada.

Iontophoretic application of dopamine (DA) to caudate neurones usually has inhibitory effects but stimulation in the nigral area of the brain stem has often been reported to have facilitatory effects on caudate neurones. We mapped nigral area sites at which stimulation would evoke field potentials and unit responses in the caudate and studied the pharmacologic responsiveness of the caudate responses in 36 adult cats paralysed and lightly anaesthetised. Of five field potential waveforms which could be elicited in caudate by nigral area stimulation, only one could be localised to the pars compacta of the nigra (SNC). None of the field potentials was affected by the DA agonist apomorphine or by neuroleptics (trifluoperazine and haloperidol). Action potentials could be elicited in 61 caudate neurones with stimulation in SNC; such effects disappeared when the stimulating electrode was moved 0.5 - 1 mm. Short-latency responses (<8 msec) tended to have antidromic properties. Longer-latency responses (>12 msec) tended to have orthodromic properties and were superimposed on the nigroneostriatal field potential. Cumulative intravenous dose-response experiments with apomorphine, haloperidol and trifluoperazine revealed an absence of drug effects in 9 of 12 neurones and non-specific, dose-unrelated effects on the remaining three neurones. We conclude that a non-dopaminergic input to the striatum, originating in or passing through SNC, is responsible for nigroneostriatal facilitation.

80 NEURAL PATHWAY MEDIATING SOMATIC EVOKED POTENTIALS IN THE CAUDATE NUCLEUS OF CATS. O. Diez-Martínez* J.A. Roig, J. Sepúlveda* and G. Vázquez-Nin* Depto. de Fisiología, Fac. de Medicina, Univ. Nal. de México, México 20, D.F.

In previous experiments it was shown that evoked responses in the caudate nucleus (CN) can be elicited by somatic and visual stimuli. However, the pathways followed by this sensory inflow were still unknown. Since light doses of barbiturate (10 mg/Kg) abolished the caudate responses but not the primary afferent responses, our search was oriented to the ascending unspecific sensory system. Small lesions placed in the lateral reticular thalamic n. supress the CN responses to radial nerve stimulation. It was possible to record somatic evoked responses in the mesencephalic reticular formation (MRF) with bipolar electrodes. The polarity of these evoked potentials reverses within 1 mm as the electrode was descended at the level of the rostral part of the n. reticularis pontis oralis. Moreover, the electrical stimulation in this zone evoked a short latency response in the n. centralis medialis and in the CN. In other experiments electrolitic lesions were performed in the exact locations where the smallest threshold to evoke responses in CN was found, and degeneration of ascending fibers was traced with the Fink-Heimer technique. Such fibers were found in centralis medialis, reticular nucleus and in both CN. These fibers enter the head of this nucleus at the same place where previous experiments showed that the somatic responses reverse their polarity.

The data suggest that the sensory information reaches the CN by at least two groups of fibers both coming from the MRF. One group passes through the n. centralis medialis and the other through the thalamic reticular nucleus.

81 QUANTITATIVE MORPHOLOGY OF MONKEY NEOSTRIATUM: ONE-WEEK OLD VS. ADULT. Marian DiFiglia, Tauba Pasik and Pedro Pasik. Department of Neurology, Mount Sinai School of Medicine, CUNY, New York, N.Y. 10029.

Light Microscopy: Dendritic spines of the various neuronal types were counted in material prepared by the Golgi method. Spiny type I neurons show an increase in the density of spines with age from 7 to 16 per 10 μm of dendrite length. In spiny type II neurons, the density decreases from 8 to 5 per 10 μm. At one week, the dendrites of aspiny neurons (types I, II and III) exhibit spine-like processes and other longer and thinner dendritic appendages up to 4 per 10 μm which are only rarely seen in the adult. Electron Microscopy: Synapses were counted and measured in 5 samples of 200 μm^2 of net neuropil, at each age. The mean number of synapses over the 5 samples increases significantly ($p < 0.025$) with age from 45 to 52. Using preestablished criteria for maturity of synaptic contacts, 28% are immature at one week. However, similar features are also exhibited by 7% of the contacts in the adult. Axospinous synapses increase significantly both in number, from 27 to 42 ($p < 0.01$), and in relative occurrence, from 59% at one week to 81% in the adult. In contrast, axodendritic synapses decrease significantly from 18 to 10 ($p < 0.01$) and from 39% at one week to 19% in the adult. Mean total cumulative length of synaptic contacts also increases significantly with age from 13 to 20 μm ($p < 0.001$) with a proportional increase in axospinous synapses from 57% to 85%, and a decrease in axodendritic synapses from 41% to 15%. Conclusions: Results indicate that the increase in number and cumulative length of axospinous synapses observed with age reflects primarily the two-fold increase in the number of dendritic spines of spiny type I neurons seen in Golgi material. The corresponding decrease in the number and length of axodendritic contacts suggests that up to one half of these synapses present at one week are precursors of axospinous contacts in the adult. Aided by N.I.H. Grants # NS-11631 and F22-NS-01639.

82 SOME ASCENDING AND DESCENDING PROJECTIONS OF THE SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA IN THE RAT. V.B. Domesick, R.M. Beckstead* and W.J.H. Nauta, McLean Hospital, Belmont, Mass. 02178.

Nigrostriatal and mesolimbic pathways have been amply documented by histofluorescence and chemical-lesion methods, but their topography is not easily demonstrated by these techniques. In our present study, autoradiography in cases of microelectrophoretic injection of ^{3}H-leucine-proline into the ventral tegmental area (VTA) and various medial-to-lateral subdivisions of the substantia nigra (SN) suggested the following conclusions. From lateral parts of SN, fibers ascend in the dorsomedial edge of the internal capsule and suprajacent subthalamus to a lateral part of the ventromedial thalamic nucleus (VM) and a large lateral region of the caudatoputamen (CP), whereas descending fibers pass ipsilaterally to the dorsolateral midbrain tegmentum including anterior parabrachial and intrabrachial regions. Fibers ascending from medial parts of SN follow a. the lateral part of the medial forebrain bundle to more medial regions of CP, and b. Forel's field H1 to a ventromedial part of VM, whereas descending fibers are distributed to a wide medial zone of the ipsilateral midbrain tegmentum. The suggested medial-to-lateral nigrostriatal topography is confirmed by the results of HRP injections in CP. Fibers from VTA could be followed forward in the medial forebrain bundle to all layers of the olfactory tubercle and to the nucleus accumbens (Acc), but their distribution also involves a wide ventral zone of CP lateral to Acc. Fibers descending from VTA pass ipsilaterally to a median and paramedian zone of the midbrain tegmentum, including the region of the median and dorsal raphe nuclei.

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83 A MULTIVARIATE ASSESSMENT OF CAUDATE NUCLEUS FUNCTION IN THE RAT.
Charles C. Duncan* and Leslie H. Hicks. Dept. Psych., Howard University, Washington, D.C. 20059.

The behavioral effects of six different caudate lesions were examined for six separate groups of rats and operated controls on four behavioral tasks: two-way active avoidance, a position discrimination task, one-way active avoidance, and passive avoidance. The evaluation of the separate experiments indicate that there were tasks specific deficits associated with specific lesion sites. In particular, the mid-ventral (MV) lesion group demonstrated a deficit on the acquisition of the passive avoidance task, a finding which replicated an earlier observation by Winocur (JCPP: 86, 432, 1974). The anterodorsal (AD), posterodorsal (PD), and posteroventral (PV) groups demonstrated marked impairment on the position discrimination task. Simple profile analysis on six of the variables from the four behavioral tasks indicated that the various lesioned animals in this study had redistributed themselves among three predominant profiles: anterodorsal (AD), mid-ventral (MV), and operated control (OPC). Discriminant analysis of the AD, MV, and OPC profiles revealed a clear differentiation among the animals assigned to these profiles by the profile analysis. Additional discriminant analyses also suggested that the rat caudate nucleus is primarily involved in the mediation of spatially dissociated cues. The appearance of animals with various lesions within specific profiles suggests there is a high degree of interdependence within the caudate for the mediation of regionally specific behaviors. The combination of these two notions is in line with principles of brain-behavior relationships originally postulated by Lashley in 1931.

84 STUDIES OF THE FIBER CONNECTIONS OF THE SUBSTANTIA NIGRA IN THE RAT USING THE METHOD OF RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE
R. L. M. Faull* and W. R. Mehler. Departments of Anatomy, Univ. of Auckland, New Zealand and Univ. Calif., San Francisco, and NASA-Ames Res. Ctr. Moffett Field, CA 94035

Our fiber degeneration studies in the rat with the Nauta and Fink-Heimer silver impregnation techniques have indicated that the substantia nigra pars reticularis (SNr) projects to the ventromedial nucleus of the thalamus (VM) and receives striatal afferents while the substantia nigra pars compacta (SNc) projects to the striatum. In order to localize more precisely the cells of origin of these projections and to investigate recent reports of nigrotectal (Graybiel & Sciascia, '75) and pallidonigral (Hattori et al., '75) connections, small (0.1 µl) injections of horseradish peroxidase (HRP) were stereotaxically placed in either the striatum, VM, superior colliculus or SN of 40 rats.

The results of these studies confirm that, in the rat, SNr cells project ipsilaterally to VM and to the superior colliculus, while SNc cells project topologically to the striatum. These results confirm Graybiel & Sciascia's ('75) nigrotectal findings in the cat; discrete injections into the caudal two-thirds of the stratum griseum mediale of the rat superior colliculus consistently producing conspicuous labelling of ipsilateral SNr cells especially in the lateral third and a ventral lamina of the pars reticularis. In 12 cases with nigral injections, clusters of medium-sized HRP positive neurons were consistently present in the ipsilateral striatum; labelled pallidal cells were only encountered in experiments where the subthalamic nucleus was obviously involved. These observations do not support the notion of a pallidonigral projection but they do confirm that, as in the cat (Grofova, '75), the strionigral pathway in the rat originates chiefly in the medium-sized striatal cells.

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85 ELECTROPHYSIOLOGICAL STUDY OF THE DISTRIBUTION OF AXONAL BRANCHES OF INDIVIDUAL ENTOPEDUNCULAR NEURONS IN THE CAT. M. Filion, C. Harnois* and G. Guano*.Neurobiology Lab., Fac. Med. Laval Univ. Quebec, QUE. Canada G1K 7P4.

Antidromic invasion of individual entopeduncular (Ent) neurons was used to determine the distribution of their axonal branches. In cats under pentobarbital anesthesia bipolar stimulating electrodes were placed in the nucleus ventralis lateralis (VL) and "centre-médian" (CM) of the thalamus, in the lateral habenula (HbL) and in the nucleus tegmenti pedunculopontinus (TPP). The recording sites were labelled by injection of fast green through the extracellular recording microelectrode. Giant soma spikes were taken as evidence that we were not recording from fibers coursing through the Ent nucleus. Responses were identified as antidromic by their fixed latencies at threshold stimulation, by their ability to follow stimulation rates greater than 300/s and by the demonstration of collision block. In these conditions, individual Ent neurons were invaded antidromically from a single, from a combination of two or three or from all four stimulation sites. Neurons at the periphery of the Ent nucleus, especially in its rostral portion, were often invaded exclusively from HbL and not from VL, CM and TPP. The latency of antidromic invasion from VL (median: 1.1 ms) is in agreement with the latency of monosynaptic IPSPs (mean: 1.5 ms) recorded by Uno and Yoshida in VL-VA neurons following Ent stimulation (Brain Res., 99: 377, 1975). Thus, our data show that Ent nucleus sends information in parallel to many recipient structures through axon collaterals. An important corollary is that electrical stimulation of any recipient structure of Ent fibers can produce axon reflexes recordable in the other recipient structures and which can be easily mistaken for a direct projection. (Supported by the Medical Research Council of Canada and the Quebec Health Sciences Council).

86 **INITIATION OF MONKEY ARM MOVEMENTS DURING GLOBUS PALLIDUS COOLING.** **J. Hore and T. Vilis. Department of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1.**

The finding that units in putamen and globus pallidus (GP) discharge prior to movement onset led to the suggestion that the basal ganglia may be involved in movement initiation (1). We have investigated this suggestion by cooling through a sheath implanted in the GP of Cebus monkeys which were trained to perform a prompt arm movement in response to a visual GO signal (reaction time task). In contrast to previous work from our laboratory, where cooling the dentate nucleus of the cerebellum produced an increase in reaction time, cooling the GP produced no consistent increase in reaction time in 4 monkeys.

However impairments were observed in movements during GP cooling, for example smaller amplitude and slower extension movements and a tendency towards oscillation. Preliminary analysis of EMG records from extension movements during GP cooling, reveals that the decreased amplitude of extension movements are in part the result of increased activity of the antagonist (biceps) muscle. In addition, during extension movements, instead of a monophasic increase in triceps activity, multiple peaks which were synchronized to the beginning of movement, were observed which corresponded to 7 Hz oscillations in the acceleration record. These EMG results could be explained if during GP cooling there is a failure to inhibit stretch reflexes during the movement. Thus stretch of biceps resulting from the extension movement would produce, via stretch reflex afferents, a simultaneous decrease in triceps activity and an increase in biceps activity. Taken together these results suggest that any discharge of GP neurons prior to movement onset may be inhibiting stretch reflexes rather than contributing a major drive to the initiation of EMG activity which produces the movement. **(Supported by NIH NS-10311; MRC MT-4465).**

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87 POSSIBLE AFFECT- AND AROUSAL-RELATED UNIT ACTIVITY IN RAT NEOSTRIATUM AND LATERAL GLOBUS PALLIDUS. James J. Keene. Dept. Physiol., Sch. Med., Univ. of Puerto Rico, San Juan, P. R. 00936.

Affect coding has been defined as opposite single cell responses lasting seconds to rewarding medial forebrain bundle (MFB) and aversive midbrain reticular (RET) stimulation, and demonstrated in unanesthetized postcollicular, pretrigeminal cerveau isolé rats, and in chronically implanted, awake rats and cats. The anatomical localization of this phenomena in intralaminar thalamus and medial pallidum suggested that an intralaminar-neostriatum-pallidum-intralaminar loop may be involved. In cerveau isolé rats, dorsal neostriatal (caudate) and lateral pallidal units have been tested with MFB and RET stimuli (0.5 sec, 40 Hz, 0.5 msec cathodal pulses), or both simultaneously, presented in random order with an average variable ISI of 20 sec. An on-line PDP 11/20 computer tabulated Initial (during the stimulus train) and Prolonged (4.5 sec post-train) responses, indicating significant effects by paired t comparisons with prestimulus firing rates. For 62 neostriatal and 40 lateral pallidal units tested thus far, Initial, short-latency responses were not generally correlated, in direction or magnitude, with the Prolonged responses to the same stimuli. Principal component factor analysis identified the following MFB-RET interactions: (1) Arousal coding factors, where all three stimulus conditions elicited similar effects, accounted for significant variance of the Prolonged neostriatal responses, mostly inhibition, and of the Initial lateral pallidal responses (where 90% of the units were excited). (2) Affect-related factors, where RET-elicited excitation was blocked by MFB stimulation in the Prolonged lateral pallidal responses, and where MFB stimuli blocked RET-elicited inhibition in the Initial neostriatal responses, were also significant. The integrative functions of the above loop deserve further study.

88 ELECTROPHYSIOLOGICAL AND ANATOMICAL ANALYSIS OF THE CORTICO-CAUDATE SYSTEM. Kitai, S.T., Kocsis, J.D., and Wood, J.I., Wayne State Univ. Sch. of Med. Dept. Anat. Morin Memorial Lab., Detroit, Michigan, 48202.

The origin of the cortico-caudate system and its relationship to the pyramidal tract (PY) were studied in nembutalized cat. Intracellular responses were recorded from sensorimotor cortex following stimulation of the caudate (Cd), ventrolateral thalamus (VL) and PY. One group of cortical neurons responded antidromically to only PY stimulation and another to only Cd stimulation. The PY responding group also received monosynaptic EPSPs from VL. Intracellular recordings were also obtained from Cd neurons. Cortical stimulation induced monosynaptic EPSPs which were not obtainable from PY stimulation. Some of these recorded neurons were identified by direct intracellular injection of horseradish peroxidase (HRP) through microelectrode. Neurons receiving monosynaptic inputs from the cortex have somata of about 15 μ giving rise to many spine laden dendrites. The axon leaves the soma and gives off a fine collateral plexus and this parent axon continues for several millimeters. Extracellular pressure injections of 50% HRP delivered stereotaxically to the rostral Cd revealed that medium size pyramidal cells, containing HRP granules, were found predominantly in layer III of the sensorimotor cortex. HRP positive neurons were also observed in the substantia nigra and the intralaminar thalamus. In conclusion, at least some neurons in layer III of the sensorimotor cortex project to the Cd independent of the pyramidal tract and their target neurons are medium size Cd spiny neurons. (This work was supported by NIH Grant 00405 and RR5384).

89 AN ANATOMICAL AND ELECTROPHYSIOLOGICAL STUDY OF THE CAUDATE PROJECTION SYSTEM. J.D. Kocsis*, R.J. Preston*, and S.T. Kitai (SPON: C.A. Fox). Wayne State Univ. Sch. of Med., Dept. Anat., Morin Memorial Lab. Detroit, Michigan, 48202.

Caudate nucleus (Cd) projection neurons were studied by electrophysiological and anatomical techniques. Intracellular recordings were obtained from the Cd neurons in cats anesthetized with pentobarbital (40 mg/kg). Recording microelectrodes were filled with a horseradiah peroxidase (HRP) solution (4% in tris buffer with 0.2M KCl) or with 3M KCl. Spike potentials with latencies of 7 msec to 14 msec were evoked in Cd neurons following SN stimulation. These potentials were considered to be antidromic according to the following criteria: 1) all-or-none constant latency response at threshold, 2) collision with a preceding spontaneous or intracellularly induced spike and 3) absence of underlying synaptic potentials. Neurons responding in this manner responded with EPSP's to thalamic, cerebral cortical or higher intensity SN stimulation. Intracellular injection of HRP into these antidromically activated neurons indicate that they are spiny neurons. In additional experiments extracellular electrophoretic injections of HRP (4% in PO_4 buffer) were applied to the entopeduncular nucleus or SN. Sagittal sections collected to control against cross-cutting large fusiform Cd cells showed a large number of small to medium sized neurons with four to six proximal dendrites filled with HRP positive granules. No large Cd neurons contained HRP granules. In conclusion, Cd projection neurons to the SN may be spiny neurons. A relatively large number of small to medium sized caudato-fugal neurons are distributed uniformly within the head of the caudate nucleus. (This work was supported by NIH Grant 00405 and RR5384.)

90 Maze Learning in Kittens with Caudate or Frontal Cortical Lesions. M.S. Levine, J. Villablanca, C.D. Hull, N.A. Buchwald and C.E. Olmstead. Mental Retardation Research Ctr., Brain Research Inst., UCLA, Los Angeles, 90024.

We have been studying the development of kittens subjected to bilateral caudate (Cd,N=10) or bilateral frontal cortical lesions (Fr,N=9) during the first month of life (9-36 days) and comparing their performance on a set of behavioral tests with that of their sham-operated (Sh,N=5) and intact littermates (Norm,N=15). Two tests begun when kittens were about 90 days old were a visual discrimination and its reversal in a walk-through T-maze. Kittens were trained to walk to the side containing a black board placed on the floor just past the choice point of the T. After reaching criterial performance of at least 80% correct for 4 consecutive sessions, the meaning of the cue was reversed; kittens were trained to walk to the side not containing the cue.

Mean trials to criterion for Norm and Sh kittens were 258 and 210 respectively. Cd and Fr kittens were impaired compared to their controls. Mean trials to criterion being 315 and 350 respectively. There was a direct relationship between the magnitude of the impairment and the amount of caudate removal. Kittens with over 60% caudate removal (N=6) took longer to learn (357 trials) than animals with smaller lesions (253 trials).

On the reversal task Cd kittens performed less well than Fr, Sh or Norm kittens. This deficit was due to a marked tendency to persevere in responding to the previously reinforced cue. The magnitude of this deficit was also directly related to the size of the Cd ablation. The results indicate that some of the effects of both caudate and cortical damage inflicted in neonates persist at least through 6 months of age with caudate damage producing more severe disturbances in reversal learning than cortical damage. Supported by Grants USPHS HD-05958 and MH-7097.

91 **OROPHARYNGEAL SENSORY INFLUENCES ON ENTOPEDUNCULAR UNIT ACTIVITY.**
T. I. Lidsky, J. H. Robinson*, F. J. Denaro*, Dept. Psychol., SUNY Stony Brook, N.Y. 11794

Considerable work has implicated the basal ganglia in the control of oro-ingestive behavior. The purpose of the present research was to describe how neuronal activity in the entopeduncular nucleus is affected by jaw movement-related sensory stimuli. Units were recorded extracellularly in awake, paralyzed and locally anesthetized cats. Cells were physiologically identified by caudate stimulation. The effects of two types of sensory input were tested. 1) Afferents from periodontal mechanoreceptors involved in reflex jaw opening were stimulated via electrodes in the inferior dental nerve (IDN). 2) Afferents from spindle receptors in jaw elevator muscles, involved in reflex jaw closure, were stimulated via electrodes in the tract of the mesencephalic nucleus of the trigeminal (MNT). A significant proportion of entopeduncular units (95 units sampled) responded to stimulation of the IDN (30%) or MNT (51%). Of responsive cells, half responded to stimulation of both MNT and IDN; ipsilateral and contralateral stimulation were equally effective. Units responsive to more than one input usually showed qualitatively similar response patterns to all inputs with mainly quantitative properties serving to differentiate stimulation loci. Responses were most typically bursts of evoked action potentials at long latency (mean latency to IDN=60 msec, MNT=85 msec). Multiple unit recording from the trigeminal nerve indicated that evoked entopeduncular unit responses occurred well after the cessation of reflexive motor responses.

92 TETANUS TOXIN AND TURNING. Patrick L. McGeer and Edith G. McGeer. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Stereotaxic injection of 1 µl of tetanus toxin into one caudate of adult Wistar rats caused rotation towards the injected side which began 4-5 days after injection and persisted for approximately two weeks. During this period, the injected rats showed continuous circling when disturbed and occasionally in the home cage; no straight or contralateral movements were observed at the peak. Even at rest, the rats were often in an extreme position of flexion with both head and tail sharply bent toward the injected side. In a small percentage of rats, there was some gnawing of the foot or tail. Otherwise, the rats appeared healthy. Ipsilateral thalamic lesions potentiated the turning. Injections into the substantia nigra (SN) or thalamus led to more violent and continual ipsilateral movements which developed within 1-2 days and led to death by the 4th-5th day post injection. Injections into the hippocampus produced no visible change in movement or behavior.

Caudate injections still produced rotation in rats previously treated with 6-OHDA but not in animals previously hemitransected on the same side; SN injections were still effective in such rats. This, plus the long latency after caudate injections as compared with SN or thalamic injections, would be consistent with transport from the caudate before action.

The phenomenon occurred with toxin which was lethal to mice within 2-3 days when 1 ml of $1/10^6$ dilution was injected i.p. but not with toxin that had lost its toxicity. This may provide both an experimental model for the determination of new neuronal defects which might cause dystonic conditions and a test system for the evaluation of phamacological agents for use in such conditions.

93 AFFERENT CONNECTIONS OF THE NUCLEUS CENTRUM MEDIANUM IN THE CAT. Charlotte McGuinness*, Mario Dalsass*, Eric Proshansky* and George Krauthamer, Department of Anatomy, CMDNJ-Rutgers Medical School, Piscataway, New Jersey 08854.

Retrograde transport of horseradish peroxidase (HRP) was used to demonstrate afferent connections of the nucleus centrum medianum (CM) in the cat. Iontophoretic delivery of HRP (30% dissolved in 0.01M saline) through micropipettes produced small dense deposits of HRP (.3-1mm diameter) which were essentially confined to CM. In cats sacrificed after twenty-four hours the largest concentration of HRP labelled neurons was found in the ipsilateral entopeduncular nucleus. HRP positive neurons were also found in ipsilateral precruciate cortex, the substantia nigra, ventral layers of the superior colliculus and the locus coeruleus. Labelled cells were found bilaterally in the periaqueductal grey and throughout the reticular formation. In animals which survived for two and three days HRP labelled neurons were further seen bilaterally in the vestibular complex and in the cervical spinal cord, in lamina VII of Rexed. This overlap of projections within CM supports the notion that this area is involved in the integration of basal ganglia functions with somatosensory, especially spinothalamic, and reticular mechanisms.

(Supported by NIH grant NS 10922.)

94 INTRINSIC ORGANIZATION OF A SINGLE CAUDATE NUCLEUS. Patricia L. Mensah, Department of Anatomy, University of Southern California School of Medicine, Los Angeles, California 90033.

Patterns of cell clustering and interconnectivity within a single mouse caudate nucleus were studied using Nissl staining, Golgi techniques and horseradish peroxidase histochemistry (HRP). For analysis, the caudate-putamen complex was divided into four quadrants: dorsomedial (dm), dorsolateral (dl), ventrolateral (vl), and ventromedial (vm). In each, clusters of neurons, usually 10 to 12 in number, often are separated by internal capsule fiber bundles. Medium sized spiny cells comprise 96% of the total caudate cell population and 95% to 100% of each of the four quadrants. These cells are stellate, giving rise to short, thin axons and to several branching dendrites profusely covered with spines. Giant fusiform neurons also occur, almost exclusively in dm, dl, and vl striatal regions, and comprise 1% of the total cell population. They are surrounded by several stellate cells. Medium-sized varicose, smooth and long-axoned cells as well as small cells are also present.

HRP was injected electrophoretically or deposited via insect pin in the caudate nucleus of normal mice or of mice subjected to unilateral cortex removal 20 to 30 days previously. Intracellular label was seen most often in medium cells lying within a 100 micron radius of the localized injection site. When more medial, and particularly vm areas, of the caudate nucleus were injected, HRP granules were also seen in dl and vl giant neurons. These data together with those of Golgi and Nissl procedures suggest that the heterogeneous caudate nucleus may be composed of specific territories of varying interconnectivity. A ventromedial association area appears to be present.

95 Kitten Caudate Nucleus Development II. Ventral Tegmental-Caudate Connections. R. Morris*, N.A. Buchwald, and D.R.G. Fuller*. Mental Retardation Research Ctr., Brain Research Inst., UCLA, Los Angeles, 90024.

Responses of caudate units to electrical stimulation of the midbrain and precruciate cortex were studied in neonatal kittens ranging in age from one to fifty days. Overall mean latencies for all units evoked in each animal by ventral tegmental stimulation were longer (e.g. 75 msec., day 3; 52 msec., day 20) than the comparable cortically evoked responses (50 msec., day 3; 33 msec., day 20). However, in some of the units which were evoked by stimulation of either site the ventral tegmental latency was shorter than that of the cortex. Additionally, cortical latencies were too long for most tegmental evoked responses to be explained by a pathway involving cortical mediation. Latencies of responses evoked by thalamic stimulation (see Fuller et al. - Kitten Caudate Nucleus Development I., this meeting) are too long for the shorter latency tegmental responses to be explained by thalamic mediation. A number of units found in all ages of neonatal kittens show very small latency variation (S.D.$\leq$ 0.13 msec. at 10 days) and follow repetitive stimulation (10/sec), suggesting a direct pathway. Threshold measurements indicate that some of the responses were evoked by stimulation of the substantia nigra. Mean latency decreases ability to follow repetitive stimulation and number of evoked units increase with age. In the youngest animals (1-10 days) very few units show stable spontaneous activity. Older animals have more units which are spontaneously active and in a few of these inhibition (200 msec. duration) has been observed usually following an initial excitation, to both tegmental and cortical stimulation. This study indicates the presence of a functional pathway projecting from the ventral tegmental to the caudate nucleus from birth.

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96 BAR PRESSING AND MAZE LEARNING IN KITTEN OPERATED CAUDATECTOMIZED AND FRONTAL CATS. Charles E. Olmstead and Jaime R. Villablanca, Dept. Psychiat., Mental Retard. Res. Ctr., UCLA, CA 90024

The bar pressing and T-maze tasks which were used to test adult operated cats (see companion abstract) were applied to adult animals with bilateral caudate (BAc, N:7) or bilateral frontal cortical (BFr, N:9) lesions inflicted during the first month of life (9-36 days). Intact (N:12) and sham operated (ShO, N:5, bilateral lesions of midline cortex and callosum) littermates served as controls. For the bar pressing, intact animals readily acquired the task in 2-8 half-hour sessions and transferred to the opposite paw in 2-4 additional sessions. All 3 operated groups showed significantly slower rates of acquisition. Significantly fewer ShO and BAc cats acquired the initial response and only 2 BFr or BAc animals reached the single alternation criterion. The BAc cats, furthermore, showed significantly slower rates of transfer, overall responding and alternation; they did not exhibit postural adjustments and could bar press and drink simultaneously. There was a direct relationship between the magnitude of the deficit and the amount of caudate removal. On the T-maze reversal, only the ShO cats produced significantly more errors. The distribution of errors differed from that seen in adult operated animals; the intact and BAc cats showed errors of anticipation while the BFr and ShO groups exhibited errors of a random nature. Although our T-maze results might suggest that there were savings in the animals receiving neonatal lesions, this must be only a tentative statement because these kittens had earlier experience in mazes. The bar press data, on the other hand, indicates that while there may be some savings in the more subtle nuances of behavior, the overall effect on performance can be just as large as when the lesions occur later in life. (Supported by USPHS Grants HD-05958, MH 07097 and HD-04612).

97 SELECTIVE ABLATION OF STRIATAL NEURONS WITH NEUROEXCITATORY AGENTS. Robert Schwarcz* and Joseph T. Coyle. Dept. of Pharmacology and Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, Maryland 21205.

Certain neuroexcitatory agents have been shown to induce degeneration of intrinsic neurons of the retina. We have explored the effects of stereotaxic injection of such neurotoxins into the rat striatum. Ten days after injection, the activity of choline acetyltransferase, concentration of acetylcholine, and the activity of the synaptosomal high affinity uptake process for choline are reduced 60-70% ($p < 0.001$) as compared to the contralateral striatum. Similarly, the activity of glutamic acid decarboxylase, the concentration of GABA, and the activity of the synaptosomal high affinity uptake process for GABA are reduced 60-70% ($p < 0.001$). In contrast, the activity of tyrosine hydroxylase is increased 47% ($p < 0.01$) and the concentration of dopamine and the activity of the synaptosomal high affinity uptake process for dopamine are unaffected. After injection, the wet weight and protein content of the striatum are the same as control. Stereotaxic injection of the non-specific toxic agent $CuSO_4$ results in a parallel decrement in the neurochemical markers for all three neuronal types. Unilateral lesion in the striatum produces sustained, spontaneous rotatory behavior toward the ipsilateral side similar to that caused by unilateral ablation of the dopaminergic nigro-striatal pathway. These results suggest that a group of neuroexcitatory agents is selectively toxic to neurons with cell bodies near the site of injection but spare axons and terminals from distant neurons. This may be a useful animal model for Huntington's chorea. (Supported by USPHS Grant DA 00266).

98 BAR PRESSING AND MAZE LEARNING IN ADULT OPERATED CAUDATECTOMIZED AND FRONTAL CATS. Jaime R. Villablanca and Charles E. Olmstead, Dept. Psychiat., Mental Retard. Res. Ctr., UCLA, Los Angeles, CA 90024.

In a two-bar lever pressing (BP) situation bilateral acaudate cats (N:8) showed specific defects consisting of difficulties in shaping the use of the paws, tendency to persist at the response producing the last reward, inability to execute two concurrent motor acts and peculiar postural adjustments. These changes contributed to slow rates of responding, markedly impaired ability to alternate and interfered with all phases of acquisition and performance. Unilaterally ablated animals (N:7) exhibited only fragments of these defects. Frontal cats (N:6; bilateral removal rostral to stereotaxic plane A22) conversely, exhibited markedly irregular behavior, inability to sustain performance and slow rates of BP but without the other response peculiarities of acaudate cats. In a standard T-maze, on spatial alternation and black-white discrimination tasks, acaudate cats showed errors of perseveration while frontal cats produced mainly randomly distributed errors. Such defects were not seen in unilaterally lesioned animals. The above deficits did not manifest any marked recovery even in cats studied for up to 16 months. Sham operated cats behave like intact animals. In conclusion: 1) stable, chronic cats with caudate removal and absence of dorsolateral cortical damage are capable of learning, retention and performance far beyond previous suggestions; 2) the discrete behavioral deficits resulting from extensive caudate or frontal lesions are different suggesting that these structures play different functional roles; 3) the deficits resulting from caudate destruction are discussed in terms of interference with high level sensory-motor processes. (Supported by USPHS Grants HD-05958, MH07097 and HD-04612).

99 THE EFFECT OF CAUDATE NUCLEUS STIMULATION ON JAW MOVEMENTS.
P. M. Weinhold*, J. W. Gustafson and T. I. Lidsky. Dept. Psychol., SUNY At Stony Brook, Stony Brook, NY 11794; Dept. Psychol., Purdue Univ., Lafayette, Ind.

This research was intended to investigate the role of the caudate nucleus in the control of jaw movements. Work was focused on movements involving jaw-opening reflexes (digastric reflex-DR) and jaw-closing reflexes (masseteric reflex-MR) in chronically prepared cats. A conditioning test (C-T) procedure was employed in which the effects of conditioning stimulation of the caudate upon test evoking of the DR and MR were assessed. When the DR and MR were evoked by stimulation of trigeminal afferents, caudate stimulation was found to have predominantly inhibitory effects. These influences were observed at C-T intervals of 20-100 msec with contralateral and ipsilateral caudate stimulation being equally effective. Similar effects were observed when the DR was evoked by stimulation of the amygdala. Conditioning stimulation of the caudate typically was inhibitory at similar C-T intervals. In contrast, when the DR was evoked by stimulation of orbital cortex, caudate stimulation had predominantly facilitatory effects upon this reflex. These effects of caudate stimulation cannot be attributed to current spread to the internal capsule; caudate stimulation at high intensity (with concomitant evidence of spread) or direct stimulation of the capsule does not produce similar effects upon jaw movements.

100 NIGRAL MODULATION OF PERIPHERAL INPUTS ON CELLS IN THE STRIATUM OF RATS.
D.H. York and S. Lentz*, Dept. of Physiology, Sch. Med., U. of Missouri, Columbia, Mo. 65201

Previous studies in the cat have demonstrated evoked spikes in the caudate nucleus following stimulation of peripheral limb nerves. The present study was undertaken to evaluate the effect of peripheral inputs on striatal cells in the rat and to determine how nigro-striatal activation may influence peripheral inputs. The study was performed on male Sprague Dawley or Wistar derived rats anesthetized with urethane. Spontaneously firing single units were recorded with a tungsten microelectrode inserted stereotaxically into the striatum. An integrator counted up to a fixed number of spikes and displayed an incremental staircase output, resetting when the fixed number was reached. Thus cell firing was evaluated over several periods consisting of fixed numbers of spikes per period. A control period was alternated with a peripheral leg stimulation period. Stimulation of the foot pads with square wave pulses (1-2 msec, 14 pulses, 1-2/sec) consistently resulted in facilitation of cell discharge over control levels. Cells were evoked at constant latencies of 35-40 msec with either single spikes or bursts of 3-4 spikes. Stimulation of the substantia nigra (SN) with square wave pulses (1 msec, 14 pulses, 1-2/sec) also consistently produced elevation in discharge over control levels. However, subsequent testing of the leg stimulus was now markedly facilitated over previous levels and may remain elevated for several minutes. This occurred in spite of no change in basal firing level of control periods which were alternated with periods of leg stimulation. This pattern of response has been consistently observed in 5 animals, on 29 cells. It suggests that nigral input may set a level of excitability for various peripheral inputs. (Supported by NSF grant 1471).

Central Autonomic Regulation

101 MEDIATION OF SPECIFIC RENAL VASOCONSTRICTOR RESPONSES TO HYPOTHALAMIC STIMULATION IN THE CAT BY THE PARABRACHIAL REGION OF THE ROSTRAL PONS.
J. R. Adair, D. G. Ward*, L. P. Schramm, and D. S. Gann. Dept. Biomed. Engr., Johns Hopkins Univ. Sch. Med., Balto., Md. 21205.

The role of the parabrachial region of the dorsal rostral pons (PB) in mediating control of renal blood flow and of systemic arterial blood pressure was investigated in 9 cats anesthetized with chloralose/urethan. Electrical stimulation through electrodes placed stereotaxically in lateral and medial positions in the hypothalamus (LH and MH) of each cat, elicited pronounced systemic arterial pressor responses and renal vasoconstrictions. Electrical stimulation through electrodes placed in PB and in ventrolateral reticular formation (VLRF) also produced responses in systemic pressure and renal flow similar to those elicited by hypothalamic stimulation. Stimulation parameters were adjusted so that renal flow responses elicited from each site were approximately of the same magnitude. All stimulation sites were stimulated four or more times in random sequence with a minimum of 2 min between stimuli. After control values were obtained for each stimulation site, a unilateral lesion was made in the PB. All sites were stimulated in the same manner as used to obtain pre-lesion controls. The changes in pressure and flow responses (expressed as % change ± S.E.M.) to stimulation are shown in the table below:

	Δ Pressure			Δ Flow		
	control	post-lesion	P	control	post-lesion	P
LH	22.3±4.4	19.1±6.5	>0.5	-51.3±7.0	-22.1±5.3	<0.01
MH	33.6±6.1	35.1±7.4	>0.5	-48.7±8.9	-17.8±10.2	<0.01

Stimulation of the VLRF, posterior to the lesion, consistently produced undiminished systemic pressor responses and renal vasoconstriction throughout the durations of the experiments. Therefore, the differential effect on pressure and flow cannot be explained as a specific sensitivity of the renal system to neural intervention. The data indicate that hypothalamic control of systemic arterial and renal vasoconstriction are mediated differently. Lesions of the PB do not affect significantly the systemic pressor response to hypothalmic stimulation but do diminish markedly the vasoconstrictor response of the kidney. Thus, the data suggest that pathways mediating renal vasoconstriction in response to hypothalamic stimulation pass through the parabrachial region whereas pathways mediating systemic vasoconstriction in response to hypothalamic stimulation must bypass this region.
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102 CARDIAC ARRHYTHMIAS PRODUCED BY ELECTRICAL STIMULATION OF THE MIDBRAIN RAPHE IN THE CAT. R.A. Gillis*, C.J. Helke*, B.L. Hamilton, and V.H. Morgenroth III. Depts. of Pharmacol. & Anat., Sch. Med. & Dent., Georgetown University, Washington, D.C. 20007

Our previous studies indicated that ventricular arrhythmias produced by digitalis are in part due to activation of serotonergic neurons in the mid-brain raphe. This suggested that excitation of serotonergic neurons would have significant effects on cardiovascular function. The purpose of the present study was to test this possibility by examining cardiovascular responses to electrical stimulation of the nucleus raphe dorsalis. Cats were anesthetized with chloralose, and ECG and arterial blood pressure were monitored. Nucleus raphe dorsalis was stimulated with a concentric bipolar electrode using 20-40 Hz, 30-300 uamp, and 1 msec duration biphasic pulses. Stimulation consistently produced increases in heart rate, arterial blood pressure, and ventricular arrhythmias. In addition, stimulation produced pupil dilatation, nictitating membrane contraction, and piloerection. Pretreatment with propranolol prevented the sinus tachycardia and ventricular arrhythmias, and pretreatment with phentolamine prevented the pressor response. Stimulation of areas in the rostral pons not associated with the nucleus raphe dorsalis and its axon groups with currents as high as 300 uamp at a frequency of 20 Hz produced little or no cardiovascular effects. Stimulation of nucleus raphe dorsalis also produced increases in 5-HIAA concentration in the hypothalamus colliculi and amygdala. Tryptophan hydroxylase activity was increased in these areas as well. These results demonstrate that activation of serotonergic neurons in the nucleus raphe dorsalis of the cat results in profound changes in cardiovascular function that are mediated by the sympathetic nervous system. These results are also consistent with the idea that digitalis may activate this region and cause ventricular arrhythmias. (Supported by USPHS and the Washington Heart Association.)

103 EFFECTS OF DIENCEPHALIC STIMULATION ON CARDIOVASCULAR FUNCTION IN THE NEONATAL PIG. P.M. Gootman, N. Gootman, N.M. Buckley, L. Crane and B.J. Buckley. Division of Pediatric Cardiology, Dept. of Pediatrics, Long Island Jewish-Hillside Medical Center, State University of New York at Stony Brook, Depts. of Physiology, Downstate Medical Center, State University of New York, and Albert Einstein College of Medicine, New York.

A study has been undertaken to locate vasoactive sites in the central nervous system, to determine the patterns of responses to stimulation of these sites, and to study effects of moderate stresses on such responses in the neonate. Cardiovascular effects of stimulation of vasoactive sites in the lateral hypothalamus and zona incerta were recorded in 25 piglets ranging in age from birth to 13 days. Experiments were performed under light anesthesia with halothane, paralysis with decamethonium bromide and artificial ventilation to maintain normal blood gases and pH. The following variables were recorded: 1) arterial pressure, 2) heart rate, 3) peripheral arterial flows (femoral, renal, and/or carotid) with non-cannulating electromagnetic probes. The classical patterns of blood pressure and heart rate response reversals to changing stimulus parameters over the frequency and/or intensity range were obtained in the older piglets. These responses were accompanied by changes in carotid and femoral flows that were parallel to the blood pressure effects, while renal flow was not. In the youngest animals, high intensity and/or frequency of stimulation resulted in increased blood pressure, heart rate and femoral flow; however, decreases of these parameters to low intensity and/or frequency of stimulation at these sites were not observed in the youngest animals. The effects of either hypercapnia or hemorrhage on these responses were then investigated in selected experiments. Hypercapnia was produced by ventilating the animal with a gas mixture containing 10% CO_2. Hypercapnia resulted in depression of responses to high frequency stimulation of diencephalic sites in all animals studied; responses to low frequency stimulation in older animals were lost or reversed. These results in neonates were unexpected in view of the findings in adult animals, in which moderate hypercapnia usually leads to augmented responses to direct diencephalic stimulation. Acute non-shocking hemorrhage was produced by removal of 5 ml aliquots of blood to a total of 15-20ml/kg. Norepinephrine (0.5 μg/kl, i.v.) was administered before and after the hemorrhage, as a test of peripheral responsiveness. Responses to diencephalic stimulation were lost in the youngest animals to all but the highest intensity of stimulation; the responses, which were not lost, were diminished in magnitude. Responses were retained but were markedly diminished in the older animals. Since responses to norepinephrine were retained, loss of responses to diencephalic stimulation was probably due to the central effects of hemorrhage. Our results indicate that the substations of the neonatal cardiovascular controlling system studied have a low safety factor to these stresses. (Supported in part by the Nassau Heart Association grant #443 and NIH NS12031).

104 EFFECT ON BLOOD PRESSURE AND HEART RATE OF ELECTRICAL STIMULATION OF ACUPUNCTURE AND NON-MERIDIAN POINTS IN ANESTHETIZED RABBITS

R. L. Kline*, K. Y. Yeung*, and F. R. Calaresu. Department of Physiology University of Western Ontario, London, Ontario N6A 5C1.

The effect of electrical stimulation of acupuncture points traditionally associated with the cardiovascular system was studied in chloralose-urethane anesthetized rabbits. Blood pressure (Pa) and heart rate (HR) were recorded during bilateral stimulation with acupuncture needles of the Ho-Ku, Tsu-San-Li, or control points in the forepaw, hindlimb, hindpaw, and tail. Points were stimulated with 2 msec pulses ranging in intensity from motor threshold to 2mA, at frequencies of 4, 40, and 100Hz; the needles were either not insulated or insulated to the tip. Pa and HR decreased significantly during stimulation of acupuncture points and of those control points which were located in areas of small tissue mass (i.e. forepaw, hindpaw, and tail). Stimulation of control points in the hindlimb (gastrocnemius) did not elicit significant changes in Pa or HR even when 2mA stimulations were used. The motor threshold for acupuncture points was significantly lower than that of any of the control points. When insulated needles were inserted at various depths in the region of the Tsu-San-Li point the decrease in Pa and HR became greater as the needle tip approached the underlying peroneal nerve. Similarly, if the needles were inserted into the hindlimb control points so as to approach the tibial nerve significant decreases in Pa and HR could be elicited. Responses obtained during stimulation of Ho-Ku or control points in areas of small tissue mass were not related to the depth of the needle. The cardiovascular responses to electrical stimulation at all points were abolished by sectioning the nerve trunk which gave rise to the nerves innervating the tissues in the acupuncture and control areas. This study indicates that cardiovascular responses may be produced by stimulation at either acupuncture points or control sites if there are nerves in the immediate proximity or if the stimulus intensity is large enough. It is concluded that the responses obtained were probably efferent components of somatosympathetic reflexes evoked by activation of somatic afferent fibers. (Supported by the Ontario Ministry of Health).

105 DIRECT HYPOTHALAMO-AUTONOMIC CONNECTIONS. C. B. Saper*, A. D. Loewy, L. W. Swanson and W. M. Cowan. Dept. Anat. and Neurobiol., Sch. Med., Washington Univ., St. Louis, Mo. 63110.

Since the initial observations of Karplus and Kreidl in 1909, the hypothalamus has been known to play an important role in the central control of autonomic activity but, until recently, no direct hypothalamo-autonomic pathways have been identified.

Following large injections of horseradish peroxidase (HRP) into the region of the nucleus of the solitary tract and the dorsal motor nucleus of the vagus in the rat and cat, and into the spinal cord of rats, cats and monkeys, we have observed a more-or-less continuous group of retrogradely labeled neurons in the hypothalamus extending from the paraventricular nucleus rostrally, through the dorsal and lateral hypothalamic areas, to the posterior hypothalamic area. Unilateral injections of a mixture of tritiated proline, leucine and lysine into the hypothalamus in the rat, cat and monkey result in the anterograde transport of label from the hypothalamus, through the region dorsal to the substantia nigra and into the lateral part of the central tegmental fields. At the pontomedullary junction the labeled projection appears to split into two components: a dorsal component which courses dorsomedially through the region of the nucleus ambiguus to the ventromedial part of the nucleus of the solitary tract and the dorsal motor nucleus of the vagus of both sides; and a ventral component which continues caudally along the ventrolateral surface of the medulla into the lateral funiculus of the spinal cord. This component terminates in the intermediolateral cell column of both sides. These observations indicate that there is a direct pathway from the hypothalamus to the autonomic centers of the brainstem and spinal cord and serve to explain many of the earlier observations on the influence of the hypothalamus upon the autonomic nervous system.

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106 MORPHOGENESIS OF SYMPATHETIC PREGANGLIONIC NEURONS (SPN's) IN RATS: A QUANTITATIVE ANALYSIS. Lawrence P. Schramm, Judith M. Stribling*, George N. Barton*, and John T. Thompson*. Dept. of Biomedical Engineering, Johns Hopkins Univ. School of Medicine, Baltimore, Md.21205.
Recently, we reported that SPN's were morphologically immature in weanling rats (Brain Res. 106:166,1976). The present study suggests that the morphogenesis of these neurons is very slow, and that a great degree of interanimal variability exists in the morphology of these neurons in rats as old as 100 days postnatal. The left celiac ganglia of inbred Wistar rats, 22,35,49,63 and 100 days old were injected with a 20% solution of horseradish peroxidase (HRP) under ether anesthesia. One to two days after injection, rats were reanesthetized and perfused with a buffered 2.5% solution of glutataldehyde. Spinal cords were fixed and sectioned in the horizontal plane, and sections were stained for HRP. The excellent filling of cell bodies and proximal dendrites of celiac SPN's with reaction product permitted determination of their morphology. At level T8 of each rat, the following morphological characteristics were quantified for all neurons containing reaction product: location and orientation of cell bodies, orientation of dendrites, structure of the somatic-dendritic junction and existence of proximal dendritic branching. These data were scanned by pattern recognition programs to determine the incidence of neurons possessing particular combinations of these characteristics in rats of different ages. An average of 256 SPN's/rat were stained. Confirming our earlier study, SPN's with mediolaterally directed cell bodies and dendrites were most common in young rats while SPN's with longitudinally directed cell bodies and dendrites were most common in older rats. Between 22 and 100 days, the incidence of longitudinal dendrites increased at least 5 fold. The incidence of transverse dendrites decreased by two thirds. Cell bodies with strongly transverse orientations constituted approximately 16% of the stained neurons in the youngest rats and decreased to less than 1% in 100 day rats. Cell bodies with strongly longitudinal orientations increased nearly 5 fold during the same period. Interanimal morphological variability increased dramatically with age. For example, morphological indices which, in rats up to 49 days old, predicted ages to within one or two weeks predicted ages to within approximately eight weeks at 100 days. One interpretation of this variability is that after a period of relatively uniform development, SPN's in different rats continue to develop at greatly differing rates. Since we do not yet have data on the morphology of SPN's in rats older than 100 days, we do not know whether the SPN's of all rats finally achieve a uniform morphology or whether the variability we observe at 100 days represents a persistent differential development. These data indicate that the morphogenesis of SPN's continues through the period of sexual maturation in rats. This period greatly exceeds that during which adult somatic behavior develops and during which basic reflex control of metabolic systems is established. The results incidate that a detailed study of the morphogenesis and physiological ontogeny of these autonomic interneurons may provide significant information on the mechanisms mediating complex, adult, autonomic behavior.

107 LOCALIZATION AND IDENTIFICATION OF VAGAL CARDIOINHIBITORY NEURONS IN THE PIGEON. James S. Schwaber and David H. Cohen. Dept. of Physiol., Sch. of Med., Univ. of Virginia, Charlottesville, VA. 22901

In specifying the anatomical pathways mediating visually conditioned heart rate change in the pigeon it was shown that the vagal cardiac innervation contributes to the response development (Brain Res., 9: 15, 1968). With retrograde degeneration material the cells of origin of these vagal cardioinhibitory fibers were then localized to the dorsal motor nucleus, predominantly 0.5-1.0 mm rostral to the obex (J. Comp. Neurol., 140: 299, 1970). The present results provide further information on this localization and establish electrophysiological criteria for identifying such neurons.

Initial experiments showed that the compound action potential of the cervical vagus has 4 major components with mean conduction velocities of 22, 10, 5 and .8 m/sec. Bradycardia was not elicited until activation of the second component whose conduction velocity range was 8-14.5 m/sec. When this component was maximal, increasing stimulating current did not further enhance the bradycardic response.

The fiber diameter spectrum of the cervical vagus, calculated from electron micrographs, was then shown to have a prominent unmyelinated fiber contingent, accounting for the slowest action potential component, and a multimodal distribution of myelinated fibers, corresponding to the three faster action potential components. Electron microscopic analysis of the major thoracic and abdominal vagal branches indicated that at least 75% of the fibers in the cardiac branches are myelinated, and 50% of these have diameters > 2.4 μ. Approximately 90% of such larger myelinated fibers found in the cervical vagus distribute to the cardiac branches, and a substantial number of these are efferent to the heart, as demonstrated by electron microscopy after nerve transection. It is this larger myelinated fiber contingent that generates the action potential component conducting at 8-14.5 m/sec and is associated with bradycardia. In contrast, the abdominal vagus consists primarily of unmyelinated and smaller myelinated fibers, the vast majority of which are afferent.

In another experimental series, field potentials evoked by cervical vagal stimulation were recorded in the dorsal motor nucleus with 3M NaCl micropipettes (1.5-5.0 MΩ). In the region anatomically described as containing cardioinhibitory cells of origin, field potentials were consistently elicited by activating the action potential component associated with bradycardia (8-14.5 m/sec). Moreover, such fields were not found in the most rostral aspect of the nucleus and were minimal at levels caudal to the cardiac representation. Single unit studies indicated that neurons in the region of the cardiac representation that are activated by fibers in the cardioinhibitory velocity range were always antidromically driven, and therefore give rise to vagal efferents. Such units were never activated by stimulation of the recurrent laryngeal nerve or of the vagus nerve caudal to the heart.

These findings confirm the previous anatomical localization of the vagal cardioinhibitory cells of origin in the pigeon and show that these cells have 2.4-4.0 μ myelinated axons that conduct at 8-14.5 m/sec. Few such fibers are found in branches other than those to the heart. Thus, the following sequence of operations allows identification of vagal cardiac neurons for physiological study: (a) electrode localization to the region of the cardiac representation using the field potential activated by the cardioinhibitory fiber contingent; (b) isolation of a single neuron that is antidromically activated by cervical vagal stimulation; and (c) demonstration that its axon conducts at 8-14.5 m/sec. We have now studied over 100 neurons meeting these criteria, and all have been found to send efferent axons to the vagal cardiac branches.

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108 SYMPATHETIC INFLUENCES ON THE OLFACTORY BULB. H.U. Aguilar-Baturoni and R. Guevara-Aguilar. Depto. de Fisiología, Fac. de Medicina, Univ. Nal. de México, México 20, D.F.

Since no information is available of the projections of cervical sympathetic fibers (CS) on the olfactory bulb (OB), some experiments were performed in cats, recording at various points of OB, while stimulating CS with single shocks. Monophasic bimodal potentials were recorded in different points of the lateral part of the olfactory bulb. They had a latency of 6.1 $\pm$ 0.2 ms and could followed the frequency of stimulation on one to one rate up to 20 Hz. Higher frequencies produced post-tetanic potentiation of the evoked responses.

It was also found that the electrical stimulation of the hypothalamus evoked responses in a similar places of the olfactory bulb as the CS did. (The characteristics of this potentials are described in another paper of this annual meeting). If during the application of iterative single shock to the CS the hypothalamus was stimulated at 40 Hz during one min the magnitude of the CS's evoked responses decreased immediately. The effect lasted for 5 min.

109 RELATIONSHIP OF THE AREA POSTREMA TO MEDULLARY CARDIOVASCULAR CONTROL. Karen L. Barnes, Carlos M. Ferrario*, and John P. Conomy. Division of Research and Department of Neurology, Cleveland Clinic Foundation, Cleveland, Ohio, 44106.

We have shown that small amounts of angiotensin II (AII) delivered to the hindbrain of the dog via the vertebral arteries produce marked rises in blood pressure and vascular resistance. These same doses are without effect when given intravenously. These pressor responses have been shown to be mediated by the area postrema (AP), a neurovascular structure devoid of a blood-brain barrier extending rostrally from the obex as the lateral wall of the fourth ventricle. Bilateral destruction of the AP selectively eliminates the centrally mediated pressor response to AII; pressor responses to the infusion of other centrally vasoactive substances are not affected. Further, local cooling of the AP reversibly eliminates the pressor effects of intravertebral AII. It is not clear whether the pressor effects of intravertebral AII are mediated by neurons within the AP, or whether dendrites from cells of adjacent medullary regions are activated directly without mediation by AP cells. Experiments in chloralose anesthetized cats have attempted to define further the significance of the AP in cardiovascular control. Electrical stimulation (0.2-0.5mA) of the AP elicits a pressor response that is of similar magnitude to those produced by intravertebral infusion of AII. Firing patterns of units in the AP and surrounding regions such as the solitary tract nucleus to electrical and chemical stimulation (AII, norepinephrine) were compared to define the relationship of the AP to adjacent cardiovascularly active regions. (Supported in part by NIH Grant HL 6835 and the Rhineberger Foundation.)

110 ELECTROLYTIC LESIONS WITH PLATINUM IRIDIUM ELECTRODES PRODUCE WEANLING RAT VENTROMEDIAL (VMN) AND DORSOMEDIAL (DMN) HYPOTHALAMIC SYNDROMES. L.L. Bernardis and Larry L. Bellinger*, Depts. Surgery and Pathology, SUNY at Buffalo, Buffalo, NY, 14215.

Weanling male Sprague Dawley rats received bilateral electrolytic lesions with Platinum Iridium electrodes and an anodal current in the VMN and DMN, resp.. Sham-operated rats served as controls. Two experiments were performed, one lasting 48 days (Experiment 1), the other 33 days (Experiment 2) after the hypothalamic operations. Both experiments showed that all parameters change in the same direction as they do when electrolytic lesions are produced with stainless steel electrodes: VMN rats showed normophagia and body weight gains, reduced linear growth and increased carcass fat. In Experiment 2, however, the VMN rats displayed a temporary (14 days) hyperphagia. The rats with DMN lesions had reduced body weights, linear growth and hypophagia but normal body composition. Plasma obtained at sacrifice of Experiment 2 showed slight, but significant hyperinsulinemia in the VMN rats ($p < 0.02$ vs controls, $p < 0.05$ vs DMN rats). The DMN rats had higher prolactin levels ($p < 0.05$ vs controls, $p < 0.02$ vs VMN rats). The data indicate that the alterations that follow VMN and DMN lesions produced shortly after weaning are due to "true" tissue destruction rather than to artefactitious effects. They suggest, therefore, that an "irritative focus" hypothesis is not required to account for the alterations that have been reported following VMN and DMN lesions with stainless steel electrodes.

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111 ADRENERGIC INHIBITION OF THE ELECTRODERMAL RESPONSE. Patricia J. Bernthal*, Joanne I. Moore and Michael C. Koss. Univ Okla. Coll. Med., Okla. City, OK 73190.

Previous investigators have suggested that circulating catecholamines depress electrodermal activity in humans (Billigheimer, Arch. Exp. Pathol. Pharmakol., 1920, 88, 172). In order to verify and quantitate this effect of catecholamines, epinephrine was administered iv to cats, and the resulting changes in this sympathetic-cholinergic system analyzed. Adult cats (2.2-4.0 kg) were anesthetized with pentobarbital. Nictitating membrane responses, blood pressure changes, and heart rate, as well as footpad skin potentials, were recorded. The right ulnar nerve was stimulated once every 30 sec with a 15-22 volt, 10-12 Hz train of 2 sec duration or with a single shock of supramaximal intensity. Intravenous epinephrine (0.3, 1.0, 3.0, and 10 µg/kg) was administered before and after phentolamine (5.0-10.0 mg/kg iv) or propranolol (0.5 mg/kg iv). Administration of epinephrine resulted in a dose dependent inhibition of the EDR ranging from 20% depression at the 1.0 µg/kg dose to 82% depression at the largest dose. Four to five minutes of asphyxia depressed the response by approximately 51%. Phentolamine antagonized the depression by both epinephrine and asphyxia, while administration of propranolol was without effect. Preliminary studies indicate that epinephrine produces a similar depressive effect on electrodermal reflex activity. Angiotensin (0.25-2.0 µg/kg iv) was relatively ineffective in inhibiting either the peripheral or reflex responses. These observations suggest that epinephrine inhibits the EDR in a dose-related fashion and that an alpha-adrenergic mechanism may be involved.

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112 RESPONSES OF SINGLE UNITS IN ANTERIOR HYPOTHALAMUS TO ELECTRICAL STIMULATION OF AORTIC NERVE IN RABBITS. Andrew L. Brickman, Marc P. Kaufman, Neil Schneiderman and Guy K. Petrik*. Dept. Psychology, University of Miami, Coral Gables, Florida 33124.

Single unit activity evoked by electrical stimulation of aortic nerve was recorded from anterior hypothalamic sites in anesthetized rabbits in order to identify hypothalamic neurons sensitive to barosensory stimulation. Nerve stimulation (10 ms duration single pulses; 10 s interpulse interval; 0.4-4.0 ma) was presented via a bipolar tungsten sleeve electrode; unit activity was recorded using a glass coated tungsten microelectrode. The criterion for identifying a barosensory unit consisted of obtaining a change in firing rate of at least 50% from the prestimulation baseline during the .8 s period following stimulation onset. Assessment was based upon 50 to 200 successive sweeps displayed as a post stimulus time histogram. Of 287 recorded units, 10 were activated and 4 were inhibited. These units were histologically verified as being in the lateral preoptic and anterolateral hypothalamic areas. Activated units had a mean spontaneous firing rate of 13.3/s with a mean peak latency from stimulation onset of 133 ms; inhibited units had a mean spontaneous rate of 16.8/s and a mean peak latency of 202 ms. Of the units responding to barosensory stimulation, patterns of activation vs inhibition were related neither to recording site nor to whether stimulation was ipsilateral or contralateral. Although mean peak latencies were relatively long, 4 units (3 activated; 1 inhibited) consistently responded with peak latencies between 26 and 60 ms. This is consistent with the view that the hypothalamus may participate in the baroreceptor reflex.

113 THE EFFECT OF ANTERO-VENTRAL THIRD VENTRICLE (AV3V) TISSUE LESIONS ON THE DEVELOPMENT OF RENAL HYPERTENSION IN RATS. M.J. Brody*, J. Buggy, G.D. Fink* and A.K. Johnson. (SPON: J. Harvey). Depts. of Pharmacology, Physiology and Psychology, Univ. of Iowa, Iowa City, Iowa 52242.

Several reports have suggested that a central action of the renin-angiotensin system plays a role in the development of renal hypertension. Based on the identification of the AV3V as a focus of central angiotensin dipsogenic and pressor mechanisms (Buggy et al., Science 190:72, 1975; Johnson and Buggy, Fed. Proc. 35:814, 1976; Hoffman and Phillips, Fed. Proc. 34: 880, 1975), the effect of small AV3V lesions (extending less than 0.5 mm into brain tissue from the ventricular wall) on the development of renal hypertension was studied in rats with unilateral nephrectomy and figure-of-eight wrapping of the remaining kidney. Blood pressure (tail-cuff) and daily water intake were monitored in sham-lesioned and lesioned groups. By 10 days post-lesion no rat required forced hydration and water intakes in the sham and lesioned groups were comparable. Two weeks post-lesion the rats were subjected to renal wrapping or a sham procedure. Lesion-sham wrapped and sham lesion-sham wrapped rats did not change water intake or blood pressure. Sham lesion-wrapped rats increased water intake by 50% and developed hypertension (30 mm Hg increase in systolic pressure). Lesion-wrapped rats did not increase water intake nor did they develop hypertension. Increased drinking in response to peripheral injection of angiotensin was not present in lesion animals but was intact in sham-lesion animals. These data suggest that an intact AV3V region may be necessary for the enhanced drinking and increased arterial pressure that is normally observed in this model of hypertension. (Supported by Grants HL14388 and GM00141, Iowa Med. Res. Coun. and NIMH RSDA 1 KO2 MH00064.)

114 SPINAL PATHWAYS FOR THE SYMPATHETIC C-REFLEX. Jin Mo Chung and Robert D. Wurster. Department of Physiology, Loyola University Medical Center, Maywood, IL. 60153.

Ascending spinal pathways for the somato-sympathetic C-reflex were studied. Cats were vagotomized under α-chloralose anesthesia and the carotid sinus was denervated bilaterally. Sympathetic unit activity was recorded from several spontaneously active preganglionic units of the cervical sympathetic trunk. Post stimulus time histograms of the unit activity were made during stimulation of the common peroneal nerve at low rates (1-2 Hz). Double or triple pulse stimulation (30-50 msec intervals) of the peroneal nerve at a high intensity elicited both the sympathetic C and A-reflexes. Only the A-reflex was present when a single pulse stimulation was applied. The sympathetic C-reflex had a latency of about 500 msec and was followed by a distinctive silent period which was shorter than that of the A-reflex. The sympathetic C-reflex was abolished by making bilateral lesions in the T12 dorsolateral sulcus area where the ascending sympatho-excitatory pathways are known to be located. The A-reflex, however, was not abolished until additional lesions were placed in the dorsolateral funiculus where the ascending sympatho-inhibitory pathways are located. These data suggest that the sympathetic C-reflex is mediated by dorsolateral sulcus area of the spinal cord. The afferent C fibers elicit the sympathetic C-reflex by activating the ascending spinal sympatho-excitatory pathways while A fibers elicit the A-reflex by activating the ascending sympatho-inhibitory pathways. (Supported by NIH Grant HL 08682.)

115 THE ACTION OF CLONIDINE ON CENTRAL SYMPATHETIC REACTIVITY IN RESERPINIZED CATS. Meredith A. Davison and Michael C. Koss. Dept. of Pharmacology, Univ. of Okla. Health Sciences Center, Okla. City, OK 73190.

We have previously reported that the sympathetic-cholinergic electrodermal response (EDR) is an effective model system for studying the effects of drugs on central sympathetic reactivity and, that clonidine inhibits central reactivity in this system in a manner analogous to its action on other sympathetic systems (European Journal of Pharmacology 37, 1976). The present experiments used the electrodermal response system to investigate the mode of action of clonidine on central autonomic activity. EDR were evoked centrally by stimulation of reactive loci in the posterior hypothalamus and peripherally by stimulation of the distal portion of either the sectioned median or ulnar nerve. In normal anesthetized cats (pentobarbital, 36 mg/kg, ip) clonidine (10-100 μg/kg, iv) significantly reduced the amplitude of the centrally evoked EDR in a dose related manner. In contrast to the control animals, the centrally evoked responses in anesthetized cats pretreated with reserpine (2 mg/kg, iv) were significantly less depressed by clonidine at similar doses. The baseline amplitude of the EDR before administration of clonidine was not significantly different in the reserpinized animals as compared to the controls. Clonidine had no effect on the peripherally evoked responses in either the control or the reserpinized cats. These results support the observation that clonidine depresses central sympathetic reactivity and that clonidine's effect may be dependent, at least in part, on the integrity of central monoaminergic systems. (Supported by MH 24083-01 and grants from the Oklahoma Heart Association)

116 EFFECT OF CHLORDIAZEPOXIDE AND α-ADRENERGIC BLOCKING AGENTS ON CARDIAC ARRHYTHMIAS INDUCED BY STIMULATION OF CENTRAL SYMPATHETIC CENTERS WITH PICROTOXIN. J.A.DiMicco* and R.A. Gillis* (SPON: K.Dretchen), Dept. of Pharmacol., Georgetown Univ., Schools of Med. and Dent., Washington, D.C. 20007

Picrotoxin (2-4 mg/kg, i.v.) causes, in chloralose-anesthetized cats, arrhythmias that are mediated by the sympathetic nervous system. These arrhythmias can be prevented by bilateral stellate ganglionectomy and by pretreatment with either chlordiazepoxide (2 mg/kg, i.v.) or α-adrenergic blocking agents (phentolamine, 5 mg/kg, i.v.; tolazoline, 1 mg/kg, i.v.). Pretreatment with propranolol (1-2 mg/kg, i.v.) does not prevent the arrhythmias. To determine the site of the antiarrhythmic effect of these antagonists to picrotoxin, recordings from cardiac sympathetic nerves were made before and after the administration of picrotoxin and during conversion of the abnormal rhythm to sinus rhythm with these agents. Picrotoxin enhanced activity in sympathetic nerves and this enhancement was associated with the development of ventricular rhythm disturbances. Chlordiazepoxide was found to convert the picrotoxin-induced arrhythmia to sinus rhythm while simultaneously depressing the increased sympathetic nerve firing induced by picrotoxin. The α-adrenergic blocking agents exhibited the same antiarrhythmic effect but did not affect sympathetic nerve activity. These results suggest that chlordiazepoxide acts in the CNS to antagonize picrotoxin while α-adrenergic blocking agents act in the periphery to antagonize picrotoxin. (Supported by USPHS and the Washington Heart Association.)

117 VAGAL SYMPATHETIC FIBERS TO THE HEART. R. A. Galosy*, J. M. Atkins*, and J. H. Mitchell*. (SPON: S. M. McCann). Univ. of Texas Health Science Center, Dallas, Texas 75235.

The contribution of peripheral cardiac nerves to changes in cardiac performance to threshold sympathetic nerve stimulation was studied in 23 anesthetized dogs in two experiments. In experiment 1, the successive left side nerve transection order was fibers from superior cervical to caudal cervical ganglion, ventral ansa subclavia, ventrolateral cardiac nerve (VLCN), ventromedial cardiac nerve (VMCN) and thoracic vagus. For experiment 2, fibers from superior cervical ganglion to caudal cervical ganglion and the ventral ansa subclavia were left intact with the transection order of the remaining nerves being vagus, VLCN and VMCN. A bipolar stimulating electrode was placed on the left ventral ansa subclavian nerve and data collected before and during threshold nerve stimulation. The results showed that threshold stimulation with nerves intact produced significant ($P<.05$) increases in left ventricular pressure (exp. 1 = 7%, exp. 2 = 5%), first derivative of left ventricular pressure (exp. 1 = 15%, exp. 2 = 17%) and heart rate (exp. 1 = 5%, exp. 2 = 4%). Left atrial pressure and stroke volume did not change significantly. The significant changes in cardiac performance were eliminated only after transection of the thoracic vagus in both experiments providing evidence that important sympathetic efferent fibers to the heart course in the thoracic vagus.

118 CARDIOVASCULAR AND RESPIRATORY RESPONSES TO ALTERATIONS IN INTRACRANIAL PRESSURE. Patricia A. Grady and Otis R. Blaumanis.* Dept. Physiol., Sch. Med., Univ. of Maryland, Baltimore, Md. 21201.

Cardiovascular and respiratory responses to alterations in intracranial pressure (ICP) were explored in lightly anesthetized mongrel dogs. Previous studies have been done using deeply anesthetized animals and required extreme increases in ICP, resulting in an all-or-none Cushing response. In the present study, ICP was increased by infusion of mock cerebrospinal fluid into a lateral ventricle of spontaneously breathing animals maintained on gas anesthesia. Arterial and cerebral venous blood gases were sampled over the time course of ICP increase.

At low to moderate ICP (17-50 mm Hg) a graded increase in mean arterial pressure(MAP) was observed instead of a 'runaway' Cushing response. An increase in rate of respiration was usually seen prior to a change in MAP. The more marked the respiratory response the less marked was the MAP change. Deeper planes of anesthesia raised the threshold of response for both respiratory and MAP responses. Low to moderate increases in ICP may result in some degree of cerebral ischemia manifested by an increase in cerebral venous PCO_2. The increased respiratory drive with the concomitant decrease in arterial PCO_2 is, to some extent, effective in compensating for an increased cerebrovascular resistance. At higher levels of ICP or deep anesthesia, the respiratory response is blocked while the increase in MAP appears to play the dominant role in restoring cerebral perfusion.

These data suggest that a central mechanism exists whereby an increase in local PCO_2 resulting from decreased perfusion pressure acts via both respiratory and vasomotor centers to compensate for the effects of cerebral ischemia.

119 CARDIOVASCULAR CHANGES PRODUCED BY ELECTRICAL STIMULATION OF SEROTONERGIC NEURONS IN THE LOWER BRAIN STEM OF THE CAT. B.L. Hamilton, C.J. Helke*, V.H. Morgenroth III, and R.A. Gillis*. Depts. of Pharmacol. and Anat., School Med. and Dent., Georgetown Univ., Washington, D.C. 20007.

Studies by Coote and Macleod (J. Physiol. 241: 453, 1974) and Neumayr et al. (Life Sci. 14: 793, 1974) indicate that electrical stimulation of serotonergic neurons in the lower brain stem of the cat produces decreases in arterial blood pressure and efferent sympathetic nerve activity. These effects are opposite to those produced by electrical stimulation of serotonergic neurons in the nucleus raphe dorsalis. The purpose of the present study was to determine whether pressor effects as well as other effects consistent with activation of the sympathetic nervous system could be elicited by stimulation of brain stem raphe. This was done by examining cardiovascular responses to electrical stimulation of raphe obscurus in cats anesthetized with chloralose with monitoring of arterial blood pressure and ECG. Raphe obscurus was stimulated with a concentric bipolar electrode using 5-100 H_z, 100-150 uamps, and 1 msec duration biphasic pulses. Stimulation was found to produce increases in arterial pressure, heart rate, and in several animals, ventricular tachyarrhythmias. In addition, stimulation produced pupil dilatation and contraction of the nictating membrane. These results indicate that stimulation of serotonergic neurons in the lower brain stem can produce excitatory effects similar to those observed with activation of nucleus raphe dorsalis. (Supported by USPHS and the Washington Heart Association.)

120 CHANGES IN BODY TEMPERATURE PRODUCED BY METHOXAMINE AND ISOPRENALINE INJECTED INTO THE CEREBRAL VENTRICLES OF THE CONSCIOUS RABBIT. D.L. Jones W.L. Veale and K.E. Cooper, Division of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

Noradrenaline is a possible neurotransmitter at nerve synapses subserving thermoregulatory pathways within the brainstem. The relative involvement of alpha-and-beta-receptors with respect to thermoregulatory responses is unclear. We undertook experiments in which the alpha agonist, methoxamine, and the beta agonist, isoprenaline, were applied directly into the cerebral ventricular system at various ambient temperatures.

New Zealand white rabbits were prepared with a stereotaxic headplate in order that injections could be made at a later date into the cerebral lateral ventricle in the fully awake rabbit. Experiments were carried out in a controlled environmental chamber with continuous air flow, and the rabbits were restrained in conventional stocks to which they had been previously accustomed. Body temperature was recorded by means of a thermistor probe inserted into the colon. Intraventricular injections were made in the volume of 150 µl and doses of 300 and 600 µg methoxamine and 50 and 100 µg isoprenaline were utilized.

The results of these experiments support the suggestion that noradrenaline acts as an inhibitory substance on both the heat production and heat loss pathways in the rabbit, depending on which pathway is activated at the time by the ambient temperature. Further, it would appear that inhibition of the heat loss pathway is largely mediated through alpha adrenergic receptors, whilst the inhibition of the heat production pathways is mediated to a large extent by beta adrenergic receptors.

This work was supported by the Medical Research Council of Canada.

121 MEDULLARY RESPIRATORY NUCLEI PROJECTING TO CERVICAL AND THORACIC SPINAL CORD OF CATS AND KITTENS. Madhu Kalia. Dept. of Physiol. and Biophys., Hahnemann Medical College, Philadelphia, PA. 19102.

The descending projection from the nucleus of the solitary tract (NTS) to the spinal cord has been demonstrated anatomically in kittens by degeneration techniques (Torvik 1957). No information is available on the anatomical connections between the spinal cord and other medullary respiratory nuclei (nucleus ambiguus NA and nucleus retroambigualis NRA), which have recently been implicated in providing a direct input to spinal motor mechanisms (Merrill 1970). In the present study, the retrograde transport method of horseradish peroxidase (HRP) has been used in adult cats and young kittens (2-4 weeks) to demonstrate the medullary nuclei that send direct projections to the spinal cord. 0.05-0.1 µl of 33% HRP was injected into the lateral funiculus of the cervical and thoracic spinal cord, where lesions had produced dense degeneration in the NTS (Torvik 1957). After 24-48 hours, the animals were perfused with 0.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M PO_4 buffer pH 7.2. Serial 40µ frozen sections were incubated for development of the reaction product and counterstained with cresyl violet. Labelled cells were found in the contralateral NTS. The number of labelled cells were not as numerous as described by Torvik in the degeneration study. In addition, labelled cells were found bilaterally in the reticular formation of the medulla (Ventral resp. group) as well as in NA and NRA. This technique using microinjections of HRP excludes fibers of transit, thus making it possible to study projections to specific spinal levels. This would also explain the relatively fewer projecting cells seen in these experiments. An earlier study involving HRP injections into the NTS did not show any labelling in the spinal cord, thus precluding the possibility of reciprocal connections. (Supported by RCDA HL 00103 and grant HL 17800 from NHLI.)

122 ANALYSIS OF THE SITE OF ACTION OF CLONIDINE UTILIZING A SYMPATHETIC-CHOLINERGIC SYSTEM. Michael C. Koss. Dept. Pharmacology, Univ. OK. Col. Med., Okla. City, OK. 73190.

The inhibitory effects of clonidine on centrally and peripherally evoked electrodermal responses was observed in pentobarbital anesthetized cats. Activation of this sympathetic-cholinergic sudomotor system was accomplished by means of electrical stimulation of three central loci (posterior hypothalamus, medulla and spinal cord) as well as both the peripheral pre- and postganglionic components. Clonidine (30 μg/kg, i.v.) reduced the centrally evoked electrodermal responses following stimulation of reactive loci in the hypothalamus and medulla. The responses were most depressed following low frequency stimulation. Similar results were observed on the EDR evoked by stimulation of the cervical cord in the spinal cat. Little effect was seen following peripheral nerve stimulation. These results demonstrate that clonidine depresses the reactivity of this sympathetic-cholinergic system at all central levels including the cervical cord.

(Supported by USPHS Grant MH 25792)

123 DESCENDING PROJECTIONS FROM THE SOLITARY COMPLEX. A.D. Loewy and H. Burton. Depts. Anat. & Neurobiol. and Physiol., Wash. Univ. Sch. Med., St. Louis, Mo. 63110

The solitary complex can be subdivided into a medial (Sm), a commissural and a ventrolateral nucleus (Svl). An intermediate n. (Int), which lies ventromedial to the solitary tract, can be demarcated medially from the more densely stained cells of the dorsal motor n. of X. Dorsomedially and laterally the cells of Int blend into the small cells of Sm and the medium sized cells of Svl, respectively.

Retrogradely labeled neurons are seen in Sm, Svl and Int in cats and monkeys (M. fascicularis) after injections of a 25% horseradish peroxidase solution into the lower cerevical and/or upper thoracic spinal cord. Most of the cells are concentrated in the region of Int bilaterally. The position of the axons from these cells was determined by correlating the location of various spinal cord lesions, which were made rostral and prior to the injections, with the distribution and number of labeled neurons. A hemisection ipsilateral to the injections nearly eliminates all labeling whereas a contralateral hemisection or selected lesions of the dorsal columns result in no change in the labeling in these nuclei. A lesion of the ventral half of the ventral funiculus and lateral and ventral halves of the lateral funiculus reduces the number of cells bilaterally. Lesions that spare the medial and ventral third of the ventral funiculus together with the medial region of the lateral funiculus cause no change. We conclude that the descending fibers from both sides of the solitary complex and Int lie in the ventral funiculus and possibly within the medial aspects of the lateral funiculus at upper cervical levels. These connections may be related to medullary respiratory and/or cardioacceleratory centers. (Supported by USPHS Grants NS 09809, NS 12751, RR 05389).

124 MEDULLARY REPETITIVELY FIRING NEURONS RESPONSIVE TO CARDIOVASCULAR STATE CHANGES. J.W.Manning and S.J.Putnam*Dept.Physiol.,Emory U.,Atl.,GA.30322

A search of the medullary reticular formation(RF)with extracellular μ-electrodes was made for repetitively firing neurons(RFNs)responsive to cardiovascular(CV) state change. Experiments were performed on ether induced,α-chloralose anesthetized cats paralyzed with gallomine triethiodide;activity of brainstem medullary neurons was monitored extracellularly by tungsten μ-electrodes(7-11μF). Following suitable amplification arterial pulse pressure, ECG,unitary response and respiratory event were stored on magnetic tape. RFNs were identified throughout the extent of the RF; a number of which were polymodal in response to afferent stimuli. A population of units located in and around the nucleus and tractus solitarius and in the lateral reticular nucleus altered their firing patterns in response to stimuli that would force a new CV state. The stimuli, both pressor and depressor were elicited by activation of the carotid sinus nerve,hypothalamic loci, and systemic injection of norepinephrine(1μg/Kg I.V.).The "steady state"activity of RFNs which did respond to at least one of the inputs had frequencies of less than 1 to 50 pps. Correlations of spontaneous firing rates with respiratory and cardiac rhythms were also performed. A characteristic of the firing patterns of these RFNs was the appearance of irregular bursts of 2-8 spikes. This bursting pattern was part of the normal firing rate and constituted a sizable proportion of the total spike count in a given time. The burst patterns were dispersed on the background of firing of the neuron and an autocorrelation of the unit activity gave optimal spike intervals. In response to a pressor or depressor stimulus a change in unit activity was reflected not only in a change in spontaneous rate but also in the optimal interspike interval. It appears that for RFNs a shift in spontaneous rate as well as characteristic spike interval parallels a CV state change. (Supported by NIH Grant HL16648-13).

125 SPINAL COMPONENTS OF SYMPATHOEXCITATORY PATHWAYS AND THEIR RESPONSE PATTERNS TO BRAIN STEM STIMULATION. R. B. McCall and G. L. Gebber. Dept. Pharmacology, Michigan State University, East Lansing, MI, 48824.

Earlier work in our laboratory (McCall and Gebber, Fed. Proc. 35: 323 Abs., 1976) demonstrated the existence of two cell types (low and high frequency firing) in the thoracic intermediolateral cell column of the cat. The spontaneous discharges of both cell types were correlated in time with the R wave of the EKG. Low frequency firing units were antidromically activated by stimulation of the cervical sympathetic nerve, and thus were classified as preganglionic neurons (PSN). High frequency firing units could not be antidromically activated, and thus were classified as spinal sympathetic interneurons (SIN). The response patterns of these neuronal types to single shock stimulation of medullary pressor and depressor sites were characterized in the present study. Medullary pressor stimulation evoked single discharges in PSN and trains of spikes in SIN. Medullo-spinal conduction velocity (calculated on basis of shortest discharge onset latency) was 3.3±0.3 m/sec in 7 SIN and 3.1±0.6 m/sec in 11 PSN. These results suggest that SIN and PSN were closely interconnected components of the same sympathoexcitatory pathway. Medullary depressor stimulation inhibited the spontaneous discharges of SIN and PSN. Early and late phases of inhibition were observed. The onset latency of early inhibition was 11±3 msec in 5 SIN and 17±4 msec in 4 PSN. These values were less than the shortest onset latencies for excitation of SIN (31±3 msec) and PSN (41±6 msec) by pressor site stimulation. This observation indicates that the early inhibitory effect of depressor stimulation was spinal in origin and mediated at the level of SIN. The late phase of inhibition (onset latency ≈ 80 msec) is believed to be mediated in the brain stem. (Supported by PHS Grant HL 13187.)

126 DIGITALIS-INDUCED CARDIAC ARRHYTHMIAS: AN EFFECT MEDIATED THROUGH CENTRAL SEROTONERGIC NEURONS. V.H.Morgenroth III, C.J. Helke*, J. Dias Souza*, B.L.Hamilton, and R.A.Gillis* (SPON: F.G.Standaert), Depts. of Pharmacol. and Anat., Georgetown University Schs. of Med. and Dent., Washington, D.C. 20007

The purpose of our study was to determine whether or not central serotonergic systems are involved in mediating the central arrhythmogenic effect of digitalis. Cats were anesthetized with chloralose and intoxicated with a continuous infusion of deslanoside (2 µg/kg/min, i.v.). Brain and spinal cord tissue samples were taken at the time of ventricular fibrillation and analyzed for 5-hydroxyindoleacetic acid (5-HIAA), serotonin (5-HT), and tryptophan hydroxylase activity. 5-HIAA was found to be increased by 103%, 181% and 199% in the hypothalamus, amygdala, and colliculi respectively. Slight, but significant reductions in 5-HT concentrations were found in these areas. Tryptophan hydroxylase activity was increased by 68%, 64% and 125% in the hypothalamus, amygdala, and colliculi, respectively. In addition, animals pretreated with the tryptophan hydroxylase inhibitor, p-chlorophenylalanine (PCPA) required significantly greater doses of deslanoside to produce ventricular arrhythmias as compared to the control animals. These results indicate that cardiotoxic doses of deslanoside produce biochemical changes in the central serotonergic system that are consistent with activation of serotonergic neurons. The data obtained from PCPA treated animals suggest that deslanoside-induced excitation of central serotonergic neurons is in part responsible for the ventricular arrhythmias produced by the agent. (Supported by USPHS and the Washington Heart Association.)

127 INTERGRATIVE FUNCTION OF THE INFERIOR COLLICULUS. John W. Patrickson, C. Ovid Trouth, James A. Holloway, Rita Brooks. Dept. of Physiol., Coll., Med., Howard University, Washington, D.C., 20059

The Inferior Colliculus (I.C.) of cats anesthetized with chloralose-urethane (40 mg/kg glucochloralose, 200 mg/kg urethane) was electrically stimulated (rectangular impulses 40/sec; 1m.sec; 4 volts) with unipolar Tungsten and steel electrodes (tip diam. 3-5µm) at millimeter intervals in three coordinates. Mapping of I.C. revealed a marked increase in ventilation (tidal volume increased 5 fold; frequency increased slightly), blood pressure and heart rate. These respiratory and circulatory responses occured simultaneously with marked pupillary dilation and urinary bladder responses. Histological control revealed that these responses were elicited from the rostral portion of I.C. in the region of the central nuclei. Surprise responses to sound include pupillary dilation (Lucy et al., 1967) changes in blood pressure, heart rate, respiration and GSR (Landis, and Hunt, 1939). The possible role of I.C. in the integration of auditory information with the "startle response" is indicated. Landis, C. and Hunt, W.A. The Startle Pattern, Farrar and Rhinehart, New York, 1939. Lucy, D.D., M.V. Allen and H.S. Thompson, Neurology, 17, 763-770. (Supported by NSF Grant HES 75-09024 and NIH Grant 1 TO 2 GM 05010-01).

128 INFLUENCE OF VAGAL AND INTERCOSTAL AFFERENTS ON RESPIRATORY PATTERN DURING SLEEP. E.A. Phillipson* and P. Muir* (SPON: Y. Israel). Dept. of Med., University of Toronto, Toronto, Ontario, M5S 1A8.

During rapid-eye-movement sleep (REM) breathing is more rapid and irregular than during slow-wave sleep (SWS), but the mechanism underlying these differences is unknown. However the strength of many reflexes is known to fluctuate during REM. Accordingly, we have investigated the possibility that sleep-induced changes in either vagal or intercostal reflex influences on breathing might account for the respiratory changes of REM. Studies were performed in four dogs with exteriorized cervical vagal loops, two of whom later underwent sectioning of T_{3-7} dorsal nerve roots bilaterally. Sleep stage was determined by behavioural and EEG criteria. During REM breathing was more rapid, shallow and irregular than during SWS and coefficients of variation of all variables were higher (0.18-0.30) than in SWS (0.05-0.10). Vagal blockade (VB), induced by cooling the vagal loops, produced comparable changes in SWS and REM: breathing became slower and deeper, but the differences between stages and the irregularity of breathing in REM persisted. Similarly dorsal root section (DRX) did not affect the REM-induced changes in respiratory pattern, and combined VB and DRX also failed to abolish the rapid and irregular breathing of REM. The results indicate that changes in respiratory control and stability during REM are not due to fluctuations in either vagal or mid-thoracic afferent activity. (Supported by Grant MA-4606, MRC of Canada).

129 EFFECT OF PROSTAGLANDIN AND BACTERIAL PYROGEN, INJECTED INTO THE HYPOTHALAMUS, ON THERMOREGULATION IN SHEEP. Q.J. Pittman, W.L. Veale and K.E. Cooper, Division of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

Fever develops when prostaglandins of the E series are injected into the cerebral ventricles or the anterior hypothalamic/preoptic area (AH/POA) of a number of species of animals. We have examined the response of sheep to direct intrahypothalamic injection of prostaglandin E (PGE). Under aseptic conditions, an array of 4 to 6 guide tubes was implanted stereotaxically into 10 female sheep so that the tips of the guide tubes were positioned bilaterally above various hypothalamic and associated structures. Following recovery from surgery, 1 µl injections were made through a 27 gauge needle lowered to the appropriate depth. Body temperatures, ear skin temperatures and respiratory rates were measured. At the conclusion of a series of experiments, injection sites were identified histologically and were found to range throughout the medial hypothalamus from the posterior nucleus anterior to the septal area and nucleus accumbens. When PGE_1 or PGE_2 (0.1 to 1.0 µg) was infused into 84 separate sites, temperatures and respiratory rates did not change in the 90 min following the injection. Infusion of bacterial pyrogen (*S abortus equi* or *E. coli*, 0.1 µg) into the AH/POA but not into other injection sites caused long-lasting fevers.

The results suggest that a) fever may develop in sheep independently of the involvement of PGE in the hypothalamus and b) the hypothalamus is relatively insensitive to the direct application of large amounts of bacterial pyrogen.

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Q.J.P. is an M.R.C. student.

130 DECREASED GASTROINTESTINAL MOTILITY FOLLOWING CENTRAL SEROTONIN DEPLETIONS. Charles F. Saller* and Edward M. Stricker. (SPON: D. L. Tomko). Psychobiology Program, Departments of Life Sciences and Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

Rats depleted of brain serotonin following intraventricular injections of 5,7-dihydroxytryptamine, with desmethylimipramine pretreatments, exhibit hyperphagia and increased growth (Science, 192: 385, 1976). Rats not affected in this way often were found to suffer from severe distention of the gastrointestinal tract which persisted for months in many animals. Gastrointestinal motility also was drastically reduced in rats depleted of serotonin by peripheral injections of para-chlorophenylalanine (PCPA). Injections of PCPA (120-360 mg/kg, i.p. or s.c.) at 0 hr, or at 0 hr and 24 hr, of a 48-hr fast resulted in significantly larger wet and dry weights of stomach, small intestine, and colon than were found in saline-injected controls. This effect is dose-dependent and appears to be related to the degree of central serotonin depletion. Furthermore, emptying of a nutritive solution or of water (3 ml, i.g.) containing trace amounts of ^{14}C-polyethylene glycol was greatly slowed by PCPA (300 mg/kg, s.c.) in 24 hr-fasted rats. Other data suggest a contribution of stress, increased sympathetic activity, and hypokalemia to the ileus observed following central serotonin depletions. (Supported by NIMH grants MH-25140 and MH-20620.)

131 ALTERATION OF MEDULLARY RESPIRATORY NEURONAL ACTIVITY BY PERIPHERAL AND CENTRAL CHEMORECEPTOR AFFERENT STIMULI. W.M. St. John and S.C. Wang* Dept. of Pharm., Columbia Univ., Coll. of P. & S., New York, N.Y. 10032 and Dept. of Physiology, Dartmouth Medical School, Hanover, N.H. 03755.

Single respiratory unit activity was recorded extracellularly from both dorsal (DRN) and ventral (VRN) medullary respiratory nuclei of vagotomized, paralyzed, and ventilated decerebrate cats. Activity from phrenic nerve rootlets was also monitored. Under hyperoxic conditions (P_AO_2 > 600 mm Hg), essentially all inspiratory and expiratory units in both the DRN and VRN exhibited a progressive increase in the number of spikes per minute (count rate (CR)) and a progressive decrease in the interval between spikes (ISI) as P_ACO_2 was elevated (35-70 mm Hg). At the lower P_ACO_2 levels, the phasic discharge pattern of some units was changed to tonic firing; other units ceased firing entirely. Diminution of P_AO_2 to normoxic levels at isocapnic caused an increased CR and reestablishment of the phasic discharge pattern of some units. While, in the majority of units, progressive P_AO_2 diminutions (120-60 mm Hg) at isocapnia caused an increase in CR and a shift in ISI to shorter intervals, a significant number of other units exhibited little change or a diminution of activity. Following carotid sinus nerve section, similar progressive alterations in CR and ISI were observed as P_ACO_2 was elevated. In contrast, however, the change from hyperoxia to normoxia now resulted in a diminished CR in almost all units. Diminutions in P_AO_2 resulted in an unaltered or lowered CR. These results imply that central chemoreceptor stimuli produce a generalized excitation of the brainstem respiratory control system whereas peripheral chemoreceptor stimuli cause a more discrete excitation of controller elements. It is further concluded that, even at normoxic levels, peripheral chemoreceptor afferents provide an excitatory input to medullary respiratory units and may serve to maintain the phasic discharge characteristics of these units.

132 CROSS-CORRELATION OF MEDULLARY RESPIRATORY NEURONES IN THE CAT. Bruce R. Vachon*and James Duffin. Dept. of Physiology, University of Toronto, Canada, M5S 1A8.

Simultaneous recordings of the spike activity from pairs of respiratory neurones in the nucleus retroambigualis of the cat, were analysed using correlation techniques. Certain models (G.C. Salmoiraghi, Ann. N.Y. Acad. Sci. 109: 571, 1963) of the organization of the brainstem respiratory neurones involved with the generation of respiratory rhythm, postulate extensive interconnections in the form of self re-exciting chains of neurones for both inspiratory and expiratory neurones. Others (E.G. Merrill, Essays on the Nervous System, eds. R. Bellairs, E.G. Gray, Clarendon Press, Oxford, 1974, p. 451; M.I. Cohen, Breathing, ed. R. Porter, J.A. Churchill, London, 1970, p. 125) have proposed models which do not require this type of interconnection.

Cross-correlation of 13 pairs of inspiratory neurones and 4 pairs of expiratory neurones showed no evidence of direct neural pathways between neurones. This lack of evidence for direct neural links casts further doubt upon the self re-exciting chain hypothesis in explaining the origin of respiratory rhythm. It should also be noted that the flatness of the cross-correlograms indicates that there is not a central driving pathway in the sense of a pacemaker driving the other members of the population via a direct pathway.

133 CHARACTERIZATION OF GROUP III AND IV MUSCLE AFFERENTS SUBSERVING A PRESSOR RESPONSE. W. H. Vance and J. H. Mitchell* (SPON: D.C. German). Depts. of Internal Medicine & Physiol., Univ. of Texas Health Sci. Cntr., Dallas, Texas, 75235.

Group III and IV muscle afferents are thought to mediate the reflex increase in arterial blood pressure, heart rate and contractile state of the left ventricle which accompanies isometric exercise. Close intra-arterial injections of capsaicin (a decylinic acid amide of vanillylamine) induces a response which has been studied in this laboratory and shown to be similar to that induced by isometric exercise. In order to study the relationship between muscle afferent response and the pressor response, single unit activity was recorded from dorsal root filaments before and after intra-arterial injections of capsaicin. Unit responses were recorded from the L7 and S1 segmental levels in nembutal anesthetized cats. Units with conduction velocities characteristic of group III (5-35 m/sec) and group IV (less than 2.5 m/sec) show an increase in firing rate during and throughout the capsaicin-induced pressor response. These same small doses of capsaicin (5-100 μg) fail to change the firing rates of group I and II afferents whose conduction velocities are in excess of 35 m/sec. These data lend support to the hypothesis that the capsaicin-induced pressor response is mediated by activation of group III and IV muscle afferents. These same fibers may subserve the cardiovascular response to isometric exercise. (Supported by NHLI Grant HL19198 and the Moss Heart Fund).

134 VISCERAL AFFERENT INFLUENCES ON RENAL SYMPATHETIC EFFERENT NERVE ACTIVITY. L.C. Weaver. Dept. Physiol., Mich. State Univ., E. Lansing, MI. 48824

Control of renal function by renal sympathetic efferent nerves may be altered by visceral afferent influences from the cardiopulmonary region or from the kidney itself. Cardiopulmonary sympathetic afferent or contralateral renal afferent influences on efferent renal nerve activity were evaluated with electrophysiological techniques in alpha chloralose anesthetized, vagotomized, sino-aortic denervated cats. Single shock stimulation of the central segments of severed left inferior cardiac or right renal nerves evoked bursts of activity in left renal sympathetic efferent nerves with onset latencies of 98 and 100-130 msec respectively. Excitation was always followed by silent periods with durations of 880 and 300-600 msec respectively. Low frequency (0.5-8 Hz) stimulation of cardiopulmonary sympathetic afferent or renal afferent nerves decreased blood pressure by 5-60 mm Hg while higher frequency (10-50 Hz) stimulation often increased blood pressure by 5-45 mm Hg. Depressor or pressor responses with associated respective decreases and increases in renal efferent nerve activity could be evoked by stimulation of cardiopulmonary sympathetic afferent nerves with intensities which activated only A fibers or both A and C fibers. This suggests that physiological activation of cardiopulmonary sympathetic afferent or contralateral renal afferent nerves may enhance or depress neural influences on the kidney. Intravenous volume expansion (15 mg/kg 3% dextran in normal saline) in cats with intact cardiopulmonary sympathetic afferent or renal afferent nerves inhibited integrated renal efferent nerve activity 15-60%. Volume expansion no longer inhibited renal nerve activity after bilateral T_1-T_5 dorsal root rhizotomy. Thus, activation of cardiopulmonary sympathetic afferent nerves by volume expansion appears to inhibit renal efferent nerve activity. (Supported in part by NIH-GRS grants to Mich. State Univ. Colleges of Osteopathic and Veterinary Medicine)

135 A COMPARISON OF APPARENT AUTONOMIC RESPONSES TO ROUTINE DENTAL PROCEDURES. G. A. West*, A. E. Bastawi*, K. H. Reid. Depts. Physiology/Biophysics and Pedodontics, UL, Louisville, Ky. 40201.

Historically, evaluation of individual autonomic functions, notably heart rate and the electrodermal response, has been used to indicate changes in arousal. The objective of this study is to compare simultaneous changes in several physiological phenomena controlled by the autonomic nervous system. Heart rate, digital pulse volume and palmar skin resistance were monitored from 47 pedodontic patients aged 6-12 years, who underwent routine dental restorations. Apparent autonomic responses were compared over successive presentations of a stimulus (i.e. high speed drilling, slow speed drilling and injections), between different stimuli (i.e. high speed drilling vs. slow speed drilling) and between different patients presented with the same stimulus. Although heart rate, digital pulse volume and skin resistance are principally regulated by the sympathetic nervous system, independent changes in the activity of each are seen. Our findings show that the patterns of responses to minor stimuli do not seem to be consistent with Cannon's concept of unified sympathetic activation. The results further suggest that changes in arousal cannot be inferred reliably from changes in any one autonomic function measured. Evaluation of several functions simultaneously appears to provide a more reliable index of changes in arousal.

136 CLOSE RELATION BETWEEN BLOOD AND CSF NOREPINEPHRINE (NE) AND THE PRESENCE OF A BLOOD-CSF BARRIER TO NE. Michael G. Ziegler*, C. Raymond Lake, Michael H. Ebert*, James H. Wood*, and Benjamin R. Brooks* (SPON: Robert W. Colburn). NIH, Bethesda, MD. 20014.

In 116 patients hospitalized for various neurologic disorders CSF was removed by lumbar puncture and blood sampled while the patients were recumbent. NE was measured in both fluids by a sensitive radioenzymatic technique. The NE level in CSF closely correlates with the plasma NE level ($r=0.83$, $p < .0001$). Levels in pg/ml of NE are related by the equation NE in CSF=0.52 plasma NE + 47. To assess the blood-brain barrier for NE, ^{14}C-NE was infused intravenously into a monkey and CSF continuously sampled from a cannula chronically implanted in the monkey's lateral cerebral ventricle. NE was separated on alumina, normetanephrine on Dowex 50-W®, vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) by thin layer chromatography. ^{14}C-NE did penetrate into CSF and reached a peak 3 hours after injection but was less than 2% of maximum blood ^{14}C-NE levels. Peak levels of ^{14}C-normetanephrine in CSF were 9% of peak blood levels and peak CSF levels of ^{14}C-VMA and ^{14}C-MHPG were less than 5% of peak blood levels of these ^{14}C-labelled metabolites of NE. One patient with a pheochromocytoma had a plasma NE level of 9,680 pg/ml and a level in his CSF of 200 pg/ml. The blood-CSF barrier for NE appears sufficiently effective so that the close relation between the patients' blood and CSF levels of NE cannot be explained by penetration of blood NE into the CSF. The rate of release of NE from the peripheral sympathetic nervous system in recumbent subjects may be proportional to NE release in the central nervous system.

Cerebellum

137 AFFERENT INPUT TO THE INFERIOR OLIVARY COMPLEX AND ITS RELATION TO CEREBELLAR FUNCTION. J. T. Brown*, V. Chan-Palay, and S. L. Palay. Harvard Medical School, Boston, Mass., 02115

The inferior olivary complex in the albino rat and macaque monkey was exposed via a ventral surgical approach through the basi-occiput. Under direct visualization, minute amounts of horseradish peroxidase (HRP) were injected under pressure through glass capillary micropipettes into the inferior olivary complex. Well localized unilateral injections were obtained without damage to other brainstem areas and without spread of HRP into the adjacent reticular formation.

After retrograde transport of HRP, labelled neurons supplying axons to the inferior olivary complex in these animals were analyzed and compared. With few exceptions, labelled neurons rostral to the olive were found ipsilateral to the injection site, the greatest number situated in the nuclei of Darkschewitz and Cajal and the adjacent reticular formation (20% of all labelled neurons in the rat). The sensorimotor cortex and pretectal areas ipsilateral to the injection site also contained a relatively large number of labelled neurons.

Labelled cells caudal to the olive, on the other hand, were largely noted contralateral to the injection site, the majority located in the dorsal column nuclei and dorsal nucleus of Clarke.

In the rat the deep cerebellar nuclei, dentate and interpositus, contained the largest number of HRP labelled neurons. Forty-seven per cent of all labelled cells occurred in the contralateral dentate nucleus, 1.4% in the ipsilateral nucleus; the interpositus nuclei contained 21% on the contralateral and 0.2% on the ipsilateral side. Together these nuclei accounted for about 69% of the total number of HRP-labelled neurons. The cerebellar neurons were predominantly small with multipolar or bipolar somata. In the dentate nucleus they were found largely in small cell regions in the medial hilus area and caudal and rostral poles. Some were present in the caudolateral columnar zone and these were somewhat larger cells. The dentate nucleus in the rat contains 5.6×10^3 neurons of which 43.8% are small. Our data show that 35% of all small neurons in the contralateral dentate and 0.6% of those on the ipsilateral side project to the inferior olive. Data from radioactive amino acid injections show that the pathway is predominantly crossed with the ipsilateral contribution coming from axons that recross in the olivary commissure. In the interpositus nuclei the labelled cells were also predominantly small and were scattered throughout the nuclear masses.

These data substantiate the role of the inferior olive as a cerebellar relay station. It receives inputs from many brain centers that also project directly to the cerebellar cortex and deep nuclei; e.g., Clarke's dorsal nucleus, the red nucleus, and the lateral reticular nucleus. Thus considerable integration occurs in parallel with the cerebellum. Moreover, the inferior olive is in a direct feedback circuit with the cerebellum. Olivary climbing fibers project to cortex and their collaterals to the deep nuclei. In turn, the olive receives crossed and recrossed cerebellofugal fibers.

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138 TOPOGRAPHY OF PROJECTIONS FROM PONS, RETICULAR NUCLEI AND INFERIOR OLIVE TO THE CEREBELLAR CORTEX OF THE RAT. R. A. Burne, M. A. Eriksson*, J. A. Saint-Cyr, and D. J. Woodward. Dept. of Cell Biol. Univ. Tx. Hlth. Sci. Ctr., Southwestern Med. Sch. at Dallas, Tx 75235.

This study was undertaken to establish to what extent a point to point relationship exist in the projections from pontine grey (PG) and precerebellar reticular nuclei to cerebellar cortex in the rat. After injections (.1-.2µl) of 30% horseradish peroxidase (HRP) into various cerebellar cortical lobules, HRP- labeled cells were localized in the PG, paramedian (PRN), lateral (LRN) and pontine tegmental reticular nuclei (NRTP), and in the inferior olive (IO). To facilitate mapping of projections, subdivisions of the PG were defined as: anterior, middle, and posterior in the rostral-caudal plane; and, paramedian, intermediate and lateral in the mediolateral plane.

Projections noted were: Vermal-lobules IV & V receive from the posterior midline PG and adjacent NRTP; VI & VII bilaterally from middle dorsolateral and anterior PG; IX bilaterally from posterior lateral and middle paramedial PG. Intermediate Zone-(1.4 mm from the midline)-II & III receive from contralateral posterior paramedial PG and adjacent NRTP; V from posterior paramedian PG and adjacent NRTP; VI bilaterally from middle intermediate PG and ipsilaterally from posterior PG. Lateral Zone (2.5-3.0 mm from the midline)-V & VI (Simplex) receive bilaterally from posterior PG and contralaterally from middle intermediate PG; VII & VIII (Crus II and Paramedian) bilaterally from paramedian PG and contralaterally from middle intermediate PG; ventral paraflocculus (Pfv) bilaterally from anterior, middle paramedian and middle dorsolateral PG, and from middle NRTP. The IO projects bilaterally to all cerebellar cortical areas studied except the Pfv where it projects contralaterally. The PRN and LRN project bilaterally to the vermis of lobules IV & V, the intermediate zone of lobules II & III (LRN only), V & VI (LRN & PRN) and the lateral zone of lobules V, VI, VII & VIII.

Our results indicate that PG does not project isotopically to the cerebellar cortex; that is, some areas of pons project to restricted areas of cerebellar cortex whereas other areas of pons project to multiple cerebellar regions. The following topography was indicated: 1) In the vermis and intermediate zone, the anterior lobules (II, III, IV & V) receive projections only from the posterior pons. 2) The vermis of lobules VI & VII, and the Pfv are the only two areas studied that receive projections from the anterior pons. 3) The intermediate zone of lobules VI & VII receives projections only from the middle pons. 4) The vermis of lobule IX, and the lateral zones (Lobulus Simplex and Crus II) receive mixed projections from middle and posterior pons. With regard to reticular nuclei and IO, a marked degree of bilaterality of projections is evident. Emerging from this analysis of topography is the implication that some cerebellar areas integrate information from a few restricted brainstem sources, whereas other regions integrate information from numerous, presumably diverse, brainstem regions.

139 CEREBELLOFUGAL PROJECTIONS FROM THE DENTATE NUCLEUS: A NEW LOOK AT THEIR FUNCTIONAL TOPOGRAPHY. V. Chan-Palay, M. Sweikhart*, C. Van Itallie* and J.T. Brown*. Harvard Medical School, Boston, Ma. 02115

Experiments were performed with minute injection of ^{35}S-methionine (0.1 or 0.05 µl) precisely placed by pressure from micropipettes into the dentate nuclei of rats and rhesus monkeys. Each of 5 rats received a single injection which encompassed one entire nucleus, and the autoradiograph material obtained after orthograde transport provided a study of the course and terminations of all dentatofugal projections in thalamus, midbrain, pons, and medulla. These include uncrossed descending fibers to medulla, and pons, crossed descending branch of the superior cerebellar peduncle and the crossed ascending limb. Terminal sites include several trigeminal nuclei, locus coeruleus, several raphe nuclei, principal olivary nucleus, RTP nucleus of the pons, the pontine nuclei, several reticular formation nuclei, red nucleus (para and magnocellular portions), oculomotor nucleus, interstitial nucleus of Cajal, and Darkschewitz nucleus. In the thalamus, labelled axons were found in CM,pf,VL,VA,LP,ML, H_1 and zona incerta. The primate experiments aimed to elucidate topography in these connections. Small injections were placed into selected parts of the dentate nucleus, unilaterally, without encroachment into the interpositus nuclei. Two monkeys had a single injection each placed into the caudal pole and midflexure zone respectively, two had double separated injections, and one had three separated injections into rostral and caudal poles and the midflexure zone. The course and projections of all labelled fibers from the injected sites were traced. Superimposition of tracings from these experiments provided the topography of dentatofugal projections to the principal nucleus of the inferior olive, the RTP, pontine nuclei, red nucleus and thalamus. The principal olivary nucleus receivesapredominantly contralateral projection with recrossed axons providing a small equivalent ipsilateral one. The dentate is represented in nonoverlapping manner on the olivary nucleus with its rostral pole at the caudal olivary boundary and vice versa. The lateral dentate periphery is represented on the ventral lamellar lip, the hilar regions on the dorsal lamellar edge. The RTP and red nucleus each have a medial to lateral topographic correspondence with the caudal and rostral dentate. The dentatothalamic topography is precise, and nonoverlapping. The intralaminar nuclei ML,CL,CL superior and paracentral nuclei receive the caudal pole projection, the intermediate portions of VA,VL,Vim,X,VPL, receive the middle dentate and the rostral dentate projects laterally up to the reticular thalamic nuclei. The hilar dentate is ventral and the lateral periphery is dorsally represented upon these thalamic nuclei. There is a segmental dentatofugal topography. The caudal dentate nucleus which bears a major columnar cell region retains medial projections throughout medulla, midbrain and thalamus and is responsible for the recrossed axons providing bilateral inputs to periventricular structures including the midbrain eye movement centers. The rostral pole provides the ipsilateral uncrossed axons to the medulla and the lateralmost contralateral projection. The functional significance of these dentatofugal projections in cerebellar control of body and eye movements will be discussed.

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140 METHYLAZOXYMETHANOL-INDUCED ABERRANT PURKINJE CELL DENDRITIC DEVELOPMENT. Thomas H. Hartkop* and Margaret Z. Jones, Department of Pathology, Michigan State University, East Lansing, Michigan 48824.

Purkinje cell dendrites develop with a specific orientation and relationship to related neurons and glia. Their dendritic spine postsynaptic membrane specialization may, in turn, require a permanent presynaptic contact by parallel fibers. To determine whether changes in the surrounding cells influence the normal development of the Purkinje cell dendrites and spine specializations, destruction of the differentiating cell layer was induced in the postnatal mouse by administration of methylazoxymethanol acetate (MAM) (0.05 ul/gm body weight) at day 0. The Purkinje cells were examined by light and electron microscopy on the 10th postnatal day. The midsagittal surface area of the cerebellar vermis in treated animals was reduced by an average of 60%. The previously reported MAM-induced granule cell depletion and Purkinje cell dislocation was corroborated by light microscopy (Jones, Mickelsen and Yang, Prog. Neuropath. 3:91-114, 1973; Jones and Gardner, J. Neuropath. Exp. Neurol. 35, 1976). When compared to controls, examination following Golgi impregnation revealed random orientation of Purkinje cell apical poles, and multiple primary dendrites of reduced length with few branches, branchlets and spines. Vertical processes of Golgi epithelial (Bergmann) cells were obliquely directed, reduced in length and complexity in MAM-treated mice. Ultrastructural examination revealed naked Purkinje cell dendritic spine specializations in both control and MAM-treated mice, similar to those previously reported at later ages in treated mice (Hirano and Jones, Fed. Proc. 31:1517-1519, 1972). Although necrotic debris persisted in astrocytes and macrophages, degenerating presynaptic terminals were not found. Demonstration of naked Purkinje cell dendritic spine specializations in both control and treatment groups at this early age suggests that permanent presynaptic contact by parallel fibers is not essential for their development. Absence of degenerating terminals supports this interpretation. Astrocytic reactions to injury, in association with the reduced folial expansion, may have contributed to the observed abnormalities and disorientation of the Purkinje cells. The data suggests that Purkinje cell dendritic development may be strongly influenced by changes in surrounding cells.

141 INTRINSIC PURKINJE CELL ABNORMALITIES IN STAGGERER MUTANT MICE REVEALED BY ANALYSIS OF A STAGGERER↔NORMAL CHIMERA. Karl Herrup* and Richard J. Mullen* (Spon: M. Schachner). Dept. of Neurosci., Children's Hosp. Med. Cntr., Boston, MA. 02115.

In the mouse cerebellar mutant, staggerer, degeneration of granule cell neurons has been suggested to occur secondary to a retarded and incomplete development of Purkinje cells (PC). Observations on staggerer↔normal (sg/sg↔+/+) chimeric mice led us to re-examine the morphology of homozygous mutants (sg/sg). Staggerer cerebella from mice 20 days or older were examined in sagittal sections. Three regions were identified in sg/sg, each with a different manifestation of the disease. In the medial vermis, the "classical" staggerer phenotype was found. The cross-sectional area was reduced and the foliation only rudimentary. As in all regions, granule cells had degenerated or were degenerating. Large neurons, easily recognizable as PCs were found but did not form a normal PC layer. In the lateral hemispheres, the cross-sectional area and the pattern of foliation was similar to the vermis but PCs were difficult to identify. More commonly, medium-sized neurons were found evenly distributed in the rudimentary cortex amidst the degenerating granule cells in 20-day-old mice. These neurons were too numerous to be Golgi II cells and presumably were ectopic PCs. Between the medial vermis and the lateral hemisphere, was an attenuated bar of tissue which formed an isthmus connecting the two structures. In this isthmus the disease expression was most severe. The cross-sectional area was most severely reduced and the foliation was non-existent or restricted to a few shallow sulci. As in the lateral hemisphere, there were few unambiguous PCs but the number of medium-sized neurons was reduced and these often appeared in clusters.

The sg/sg ↔+/+ chimera which led to the above re-examination contained a cerebellum with intermediate foliation patterns throughout the cerebellum--better demarcated than in sg/sg but missing the definition and size of the wild-type cerebellum. Well-defined molecular, PC, and internal granule cell layers were present, the latter in sharp contrast to the homozygous mutant. The most striking defect was in the PC layer. In the region between vermis and hemisphere, corresponding to the isthmus in sg/sg, there was a striking deficiency of PCs, with large gaps totally devoid of PCs. Using beta-glucuronidase as an independent cell marker, it was discovered that virtually all of the remaining PCs in this region were of the +/+ genotype (analysis unfortunately was not performed on all sections from medial vermis). In the lateral hemisphere, unusually large numbers of medium-sized neurons were found in the granule cell layer resembling the PCs in sg/sg. These occurred predominantly in the gaps between the +/+ PCs and their genotype, as revealed by glucuronidase histochemistry, was sg/sg.

These observations lead to the following conclusions: 1) in sg/sg, the PCs are more severely affected than just being smaller in size and lacking tertiary branchlet spines: they are reduced in numbers and are, in many regions, ectopic; 2) these defects are intrinsic to the PC itself, otherwise the normal appearing PCs in the chimera would be a mixture of both +/+ and sg/sg genotypes; 3) in sg/sg, the effect of the mutation on PCs varies in different cerebellar regions; 4) this regional pattern must also be intrinsic to the PC for it too is conserved in the sg/sg cells in the chimera despite the presence of a relatively normal cortical structure. The sg/sg PCs are, therefore, sensitive to their location in the cerebellum and manifest the phenotype characteristic of that location even in the relatively normal cortex of the chimera. This work re-emphasizes the power of chimeras in the analysis of neurological mutants and of cellular interactions in the developing mammalian nervous system.

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142 ALTERATIONS OF VESTIBULO-OCULAR AND OPTOKINETIC EYE MOVEMENTS FOLLOWING LESIONS OF THE DORSAL CAP OF THE INFERIOR OLIVE IN RABBITS. J.I. Simpson* and N.H. Barmack, (SPON: R.S. Dow). Div. Neurobiol., Univ. of Iowa, Iowa City and Neurological Sciences Inst., Good Samaritan Hospital & Med. Cntr. Portland, Or.

The climbing fibers which originate from the dorsal cap of the inferior olive and synapse on Purkinje cells of the contralateral cerebellar flocculus are maximally activated by large field visual stimuli which move in the posterior-anterior direction at 1 deg/sec. Since the flocculus Purkinje cells make monosynaptic inhibitory connections on the ipsilateral vestibular nuclei, the olivo-cerebellar system is strategically located for altering vestibulo-ocular (VOR) and optokinetic (OKN) reflexes. We have studied the effects of discrete, unilateral, electrolytic lesions of the right dorsal cap on VOR and OKN reflexes. The sites of the lesions in the right dorsal cap were determined physiologically on the basis of the maximal amplitude field potential evoked by photic stimulation of the left eye in sodium pentobarbital anesthetized rabbits. Lesions were made by passing a 20-50 μa for 15-30 sec electrode negative. The extent of the lesions was determined histologically. The immediate effect (5 hours post-op) of selective destruction of the right dorsal cap was to cause a slow (less than 2 deg/sec) conjugate drift of the eyes toward the left with resetting saccades toward the right when the animal was placed in total darkness. The gain of the VOR (Right peak velocity + Left peak velocity)/2(Peak table velocity), in the dark was not changed by dorsal cap lesions. However, there was a marked asymmetry in the velocity of eye movements evoked by sinusoidal oscillations of the rate table in the horizontal plane at frequencies of .01-.6 Hz and amplitudes of $\pm$10 deg. This velocity bias, (Right peak velocity - Left peak velocity)/(Right peak velocity + Left peak velocity) was always toward the left and reached values of -.8 at low frequencies of oscillation (.05 - .1 Hz). We attribute this deficit to asymmetric spontaneous climbing fiber activity created by the lesion in the right dorsal cap.

Monocular sinusoidal OKN stimulation with large (70 deg) contrast rich stimuli presented to the left eye of normal rabbits evokes conjugate eye movements with a higher velocity towards the right. However, following destruction of the right dorsal cap, the rightward velocity bias of the OKN reflex was decreased and at some frequencies even reversed. The retinal slip velocity at which the decrease or reversal of the normal OKN bias was maximal corresponded to the velocity at which climbing fibers have their maximal directional selectivity. Further testing with constant velocity OKN stimuli delivered to the left eye demonstrated a decreased OKN gain to the right, but an increased gain to the left.

These data indicate that visual climbing fibers constitute part of a feedback loop which corrects for low velocity drifts of the visual field on the retina and that each dorsal cap is maximally sensitive to drifts of the visual field in the posterior-anterior direction. These data further suggest that climbing fiber activity of the right dorsal cap increases the activity of the left vestibular nuclei indirectly by reducing the net inhibitory output of the left flocculus, and/or directly by exciting vestibular neurons through axon collaterals.

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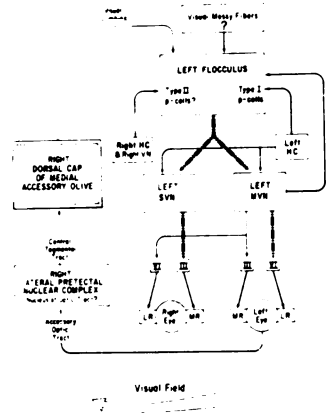

143 HISTOLOGICAL MATURITY OF THE DEVELOPING MAMMALIAN CEREBELLUM AND THE APPEARANCE OF CEREBELLAR D-AMINO ACID OXIDASE ACTIVITY. William R. Weimar and Allen H. Neims*. Roche Developmental Pharmacology Unit, Dept. of Pharmacology, McGill Univ., Montreal, Canada.

D-Amino acid oxidase (D-AAO) is highly localized in rhombencephalic regions of the mammalian CNS with the highest levels in the cerebellar cortex (Cb) (Neims et al, J. Neurochem., 13, 163(1966)). There is a striking phylogenesis of the regionalization of D-AAO activity in the CNS of various vertebrates (Goldstein, J. Neurochem., 13, 1011(1966)). Utilizing both peroxidase-coupled histochemistry and immunofluorescence, we have localized D-AAO in many mossy fibers and their terminals (Cb glomeruli) in adult hog cerebellum. D-AAO was also prominent in a cell type around the Purkinje cell (PC) soma. Associated with this cell type were D-AAO containing fibers in the molecular layer (ML) which extended to the pial surface. D-AAO histochemistry of adult human Cb revealed a pattern of staining very similar to that of hog Cb. In other mammalian species examined (cat, dog, mouse and rat) glomerular staining was less intense than that observed in hog or man, while staining around the PC soma, in the ML and at the pial surface was more pronounced in these species. These results suggest that (1) mossy fibers and their terminals and (2) a cell type of cerebellar origin contribute to total Cb D-AAO activity in varying proportions depending on the species.

The ontogenesis of Cb D-AAO activity in several mammalian species is consistent with the above interpretation. D-AAO activity in rat Cb is biochemically undetectable (<2 nmoles D-allo-hydroxyproline oxidized $min^{-1}g^{-1}$wet wt.) until postnatal day 14. D-AAO then rises to essentially adult levels by day 29 (299 ± 10(♂) (day 29 rat Cb) vs 372 ± 54 ('adult' rat Cb, day 94)). D-AAO containing cells were observed histochemically in the external germinal zone (EGZ) and ML of rat Cb vermis beginning day 15. These cells acquire D-AAO while still in the EGZ and migrate to their final destinations in the Cb cortex. In adult rat Cb, strong staining is observed around PC soma and at the pial surface. A gradient in intensity of this pattern of staining was observed in 21 day rat Cb with the most intense staining observed at the depth of the sulcus. These results suggest that a D-AAO containing cell type is among the last cells to form from the EGZ and that these cells leave processes at the pial surface and migrate to a position around the PC soma. Histological events including dissolution of the EGZ and maturation of Cb glomeruli occur at similar ages in rat and mouse Cb. It is of interest that the ontogenesis of D-AAO in mouse Cb was indistinguishable from that in rat Cb; i.e. no activity until day 14-15 then a rapid rise to adult levels by day 30.

At birth, guinea pig Cb is very mature histologically and electrophysiologically as compared to rat or mouse Cb. The ontogenesis of D-AAO in guinea pig Cb was found to occur almost entirely before birth. Guinea pig Cb from 53, 58 and 60 gestation day litters (15, 10 and 8 days before normal parturition respectively) contained no detectable D-AAO. D-AAO then rapidly rose to adult levels by 3 days after birth, with near adult levels achieved by 68 days gestational age. Histological studies indicated that the EGZ had dissipated in most areas of guinea pig Cb by postnatal day 3.

Developmental histological, histochemical and biohemical data in several species suggest that D-AAO is associated with one of the last cell types to form from the EGZ and possibly with development of glomeruli. The parallel phylogenesis and ontogenesis of D-AAO in rhombencephalic derivatives of vertebrate CNS suggest presence of functional system(s) characterized by cell type(s) containing D-amino acid oxidase.

144 PROJECTIONS FROM THE PERIHYPOGLOSSAL COMPLEX TO THE VESTIBULOCEREBELLUM OF THE RABBIT. K. Alley, R. Baker and J. Simpson*.
Dept. of Anat., Case Western Reserve Univ., Cleveland, Ohio and Div. of Neurobiol., Univ. of Iowa, Iowa City, Iowa.

In an earlier analysis of the inferior olivary source of visual climbing fibers to the vestibulo-cerebellum we noted in passing that the perihypoglossal complex provided an input to the flocculus and nodulus. Since relatively little is known about the anatomical organization of this nucleus, a more detailed examination of the projections of these neurons to the cerebellar flocculus has been initiated. We have studied this pathway by means of a combination of orthograde and retrograde axon tracer technology in adult rabbits. Micropipettes filled with either horseradish peroxidase (HRP, 30% in 0.9% saline) or an H^3-amino acid mixture of proline and leucine (25 μC/μL) were used to inject small amounts of tracer into either the flocculus or the perihypoglossal nuclei. Injections of HRP into the flocculus gave a positive peroxidase reaction in neurons of all three components of the perihypoglossal complex. These results were further verified by placing H^3-amino acid directly into the perihypoglossal nucleus. Axons from this cell group were followed into the flocculus where they ended in the granule cell layer, thus suggesting that they terminate as mossy fiber rosettes. The available anatomical and physiological evidence suggests that the perihypoglossal neurons modulate ocular motor activity both directly via projections to the eye motoneurons and indirectly by the influence of the flocculus on the vestibulo-ocular reflex. (Supported by USPHS grant EY-01074).

145 EFFECTS OF FLOCCULAR LESIONS ON OPTOKINETIC AND VESTIBULOOCULAR REFLEXES. N. H. Barmack, Neurological Sciences Insti., Good Samaritan Hosp. & Med. Cntr., Portland, Or.

Primary and secondary vestibular afferents originating from the left labyrinth project as mossy fibers to the left cerebellar flocculus where they converge with visual climbing fiber inputs from the right dorsal cap of the inferior olive which originate from the left eye. The effect of unilateral suction ablations of the left flocculus on vestibuloocular (VOR) and monocular optokinetic (OKN) reflexes has been examined in rabbits. Horizontal VOR reflexes were assessed by oscillating the animal sinusoidally (±10 deg, .05 - .7Hz), and recording with an infrared technique the position of both eyes in the dark. Monocular OKN reflexes were assessed by exposing the left eye to a contrast rich pattern (70 x 70 deg) whose position was sinusoidally modulated on a transilluminated tangent screen (±10 deg, .005 - .2Hz); recording the position of the right eye. The immediate effect (15 minutes post-op) of the left floccular lesions was a profound conjugate nystagmus with the slow phase toward the right, when the animal was in darkness. Flocculus lesions did not reduce the OKN gain. (Gain = (Right peak velocity + Left peak velocity)/2(stimulus peak velocity). However the OKN bias toward the right was much greater than normal. (Bias = (Peak right velocity - Peak left velocity)/(Peak right velocity + Peak left velocity). The gain and bias of the VOR was influenced similarly. The bias of the eyes toward the right was most dramatic at peak VOR stimulus velocities of 2.0 deg/sec and was virtually absent at velocities greater than 30 deg/sec. These data suggest that visual and vestibular inputs are used by the flocculus in the regulation of low velocity eye movement, and that the rightward velocity bias following destruction of the flocculus could be attributed to the removal of Purkinje cell inhibition from the left vestibular nuclei. (Supported by PHS Grant EY-00828)

146 A QUANTITATIVE AUTORADIOGRAPHIC STUDY OF THE EFFECTS OF POSTNATAL UNDERNUTRITION ON THE RAT CEREBELLAR CORTEX. David E. Barnes* (SPON: L. Pellegrino). Dept. Biol. Sci., Purdue Univ.,W.Lafayette, IN 47906.

Numerous studies have shown that postnatal undernutrition results in a permanent reduction of rat cerebellar microneurons. By quantitative light microscopy and thymidine autoradiography the formation and fate of cerebellar microneurons was investigated. Rats were undernourished by feeding dams 50% of the ad libtum food intake and cross-fostered at birth. Animals were injected at 10 days of age with tritiated thymidine (10 μCi/gbw, 6.7 Ci/mM) and allowed to survive for 2,10,18,26,or 34 hours and 3,5,7,9, or 11 days. The number of labeled cells and total cells was determined in the external germinal, molecular and internal granular layers of lobe VIII at all ages. By 10 days of age body and brain weights were reduced 50% and 20%, respectivelt, in the undernourished animals; retardation remained constant to the time of weaning. In a similar manner, the cell deficits of the external germinal, molecular and internal granular layers of the undernourished group(20-30%) remained constant between 10 and 21 days. At two hours after injection of tritiated thymidine the ratio of labeled cell to total cells(labeling index) was equal in both groups. However, the undernourished group had a significantly higher number of grains per labeled cell. Similar results were found in animals injected at 4,6, or8 days and allowed to survive for either 2 or 6 hours. The increase in labeled cells was lower in the undernourished group and they remained longer in the external germinal layer(EGL). Although the EGL persists longer in the deprived animals, significant compensatory cell proliferation did not take place. The loss of cerebellar microneurons during undernutrition can be attributed primarily to a reduction in their germinal cell number during early postnatal development.

(Supported by a NIMH fellowship)

147 PROCESSING INFORMATION CARRIED IN A HIGH FREQUENCY WAVE: A STUDY OF THE PROPERTIES OF CEREBELLAR UNITS OF A HIGH FREQUENCY ELECTRIC FISH. Konstantin Behrend Neurobiol.Unit,SIO &Dept.Neurosc.,UCSD,La Jolla,Cal.92093

Cells in the caudal lobe of the corpus cerebelli of the high frequency electric fish Apteronotus albifrons(Gymnotoidae)are known to process information about objects that locally deform the electric field broadcasted by the fish.The response of these units was investigated by introducing a disturbing electric signal with a small diference in frequency(ΔF)to the fish's own electric organ discharge(EOD),which causes beats in the EOD.The response to an object moving alongside the fish's body deteriorates at beat frequencies >0Hz up to about 10Hz and reapears above 10Hz.Analysis of the firing pattern of the cells to the beats of the EOD reveals that at the cerebellar level of central processing the system has the properties of a band pass filter with the pass band between >0Hz and ca.10Hz.Considering the EOD as a high frequency carrier a model is proposed,showing that the information conveyed in amplitude or phase modulations of the carrier is converted by the receptors,which are tuned to the carrier, into a frequency modulated spike train traveling along the primary afferent fibers and recovered by subsequent low frequency band pass filters at different stations in the CNS,the cerebellum seeming to be a final one.

148 ASCENDING AND DESCENDING PROJECTIONS TO THE INFERIOR OLIVE OF THE CAT. Bishop, G.A., R.A. McCrea and S.T. Kitai. Wayne State University, School of Medicine, Department of Anatomy, Morin Memorial Laboratory, Detroit, Michigan.

Afferent projections to the Inferior Olive (IO) of the cat were studied using the technique of retrograde transport of Horseradish Peroxidase (HRP). Following pressure injections of a 50% solution of Type VI HRP (Sigma Co.) into the IO of 6 young adult cats, standard histological procedures for processing HRP were carried out. Sagittal sections of the pericruciate cortex and cerebellum and transverse sections of the thalamus, mesencephalon, pons and medulla were cut at 50μ and examined for retrograde uptake of HRP. In the cerebral cortex, medium and large pyramidal cells in Layer V of the anterior sigmoid gyrus and coronal gyrus bilaterally were labeled with HRP positive granules. A few scattered medium sized pyramidal cells were found in the posterior sigmoid gyrus bilaterally. Labeling was found in the nucleus subparafasicularis of the ipsilateral thalamus and a few scattered cells were found in the Fields of Forel bilaterally. In the mesencephalon ipsilateral to the injection, retrograde labeling of cells was found in the pretectal area, the lateral and middle tegmentum, the interstitial nucleus of Cajal, the nucleus of Darkschewitsch, and the ventral periaqueductal grey. Contralaterally, uptake was seen in the deep tectum, the lateral and middle tegmentum and the ventral periaqueductal grey. Cells in the contralateral gracile and cuneate nuclei were found to contain HRP positive granules. Cerebellar projections arose from small to medium sized neurons in the contralateral deep cerebellar nuclei. (This work was supported by NIH Grant 00405 and RR 5384.)

149 CEREBELLAR NUCLEO-CORTICAL PROJECTION IN THE RHESUS MONKEY. J.R. Bloedel, H. Bantli, and D.L. Tolbert. Depts. Neurosurg. and Physiol., U. of Minn. Minneapolis, Mn. 55455

Recently, a projection from the cerebellar nuclei to the cerebellar cortex was demonstrated in cats. Experiments using both horseradish peroxidase and tritiated leucine techniques demonstrated that axons from neurons in the cerebellar nuclei project topographically to the cerebellar cortex. Electrophysiological experiments demonstrated that some of the axons of this projection are collaterals of efferent fibers leaving the cerebellum via the brachium conjunctivum. To show that a cerebellar nucleo-cortical projection is also present in sub-human primates, neuroanatomical and electrophysiological experiments were performed in four Rhesus monkeys. Following an injection of tritiated leucine into the interposed nucleus, silver grains were observed overlying labeled axons projecting from this nucleus towards the cerebellar cortex. These labeled fibers were observed to enter the granular layer where they appeared to terminate. No autoradiographic grains above background levels were observed in the molecular layer. Similar to the electrophysiological observations in the cat, neurons in the dentate and interposed nuclei were activated antidromically by stimuli applied to the cerebellar surface. Interestingly, stimulation of the vermal region of the cortex antidromically activated neurons in the dentate nucleus, suggesting that the cerebellar nucleo-cortical projection originating from the dentate nucleus may not be restricted to the neocerebellar cortex. Results from preliminary experiments suggest that the nucleo-cortical fibers may be collaterals of cerebellar efferent axons projecting toward the red nucleus and/or the ventral thalamic nuclei. This work was supported by NIH Grant NS-09447-05 and Contract NO-1-NS-2332.

150 DEFICIT IN OBJECT DETECTION (ELECTROLOCATION) FOLLOWING INTERRUPTION OF CEREBELLAR FUNCTION IN THE WEAKLY ELECTRIC FISH Apteronotus albifrons. Rocco A. Bombardieri and Albert S. Feng* Dept. Neurosciences, Sch. Med., Univ. Cal. San Diego, La Jolla, CA 92093

Previous physiological evidence demonstrates that neural units in the cerebellum of weakly electric gymnotid fish (Apteronotus and Eigenmania) respond to objects which distort the fish's electric field. The nature of these responses is consistent with the idea that the caudal lobes of the cerebellum function in processing electrosensory information related to object detection. In order to behaviorally test that hypothesis, a response, unconditioned deceleration of respiration to moving objects, has been developed. This behavioral test is employed before, during, and after cooling of the cerebellum in Apteronotus albifrons. During cold block of the cerebellum object stimuli fail to elicit deceleration of respiration whereas before and after cerebellar cold block strong responses are obtained. These data provide behavioral evidence that the caudal lobes of the cerebellum in gymnotid fish are important in processing sensory information relevant to object detection mediated by the electrosensory system. Results of lesion experiments support the above finding. (This research was supported by an NIH Individual Research Fellowship to R.A. Bombardieri, NIH and NSF Research Grants to T.H. Bullock, and NASA and NIH Research Grants to S.O.E. Ebbesson)

151 THEORETICAL CONTRIBUTIONS OF CEREBELLAR ANTERIOR LOBE MOSSY FIBER SYSTEMS TO LOCOMOTOR COORDINATION IN CATS. C.C. Boylls, Jr. Dept. of Biophysics and Theoretical Biology, University of Chicago, Chicago, IL 60637.

A cat's cerebellar anterior lobe appears to be partitioned into a collection of corticonuclear compartments, each comprising a unique cerebellar (or vestibular) nuclear region together with a sagittally and mediolaterally delimited "wedge" of cerebellar cortex selectively inhibiting that region. Feedback loops involving the pre-cerebellar reticular nuclei, along with other mechanisms, allow non-trivial interactions among nuclear regions in different compartments. Mathematical analysis of these interactions under the spur of different types of spinocerebellar and spinoreticulocerebellar, mossy fiber inputs suggests the following:

1. Mossy inputs with compartmentally restricted cortical termination zones (e.g., DSCT, CCT) alter the ratios of nuclear activity among mediolaterally adjacent compartments for prolonged periods of time (seconds).
2. On the other hand, fibers terminating diffusely within many compartments (VSCT, RSCT, spino-reticulocerebellar tracts) provoke very phasic, rate-sensitive nuclear responses distributed uniformly over all affected regions.

The anterior lobe may thus yield responses having radically different time courses to mossy inputs of differing spatial extent--responses which, during locomotion, will superimpose: The prolonged alterations in compartmental nuclear activity ratios engendered by punctate mossy fiber volleys become a "background" level upon which phasic, diffuse mossy responses ride. If the diffuse mossy systems report fundamentally upon activity in intrinsic spinal locomotor circuits, while the punctate respond to peripheral events, the VSCT-like pathways could induce, via the anterior lobe, a phasic, feed-forward "speedup" in muscle force development. But the muscle groups to which the speedup is applied will be governed in part by extended biasing elicited by DSCT-like systems responding to peripheral cues.

152 INHIBITION OF HARMALINE TREMOR BY DIAZEPAM: EVIDENCE FOR A SPINAL CORD MECHANISM. Liliane Busby* and Yves Lamarre. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec.

Harmaline causes tremor by increasing the activity of the climbing fiber input to the cerebellar cortex. Mao et al. reported that harmaline tremor is associated with an increase of the cGMP content of rat cerebellum and that diazepam antagonizes both the tremor and the increase of cGMP (Brain Res., 83: 516, 1975). These authors postulated that "the burst discharges of Purkinje cells elicited by the climbing fiber activation caused by harmaline could be blocked by diazepam". The present experiments were performed to verify this hypothesis.

Using decerebrate, non-anesthetized cats, recordings were obtained from cerebellar Purkinje cells, nucleus gigantocellularis, spinal ventral roots and quadriceps muscles. Both threshold (1.0 mg/kg, i.v.) and supramaximal doses of harmaline (5.0 mg/kg) were used to induce the tremor. Diazepam at 1.0 mg/kg, i.v., abolished rhythmic activity in ventral roots and muscles without blocking the rhythmic bursting of Purkinje and reticular cells. Higher doses of diazepam (5 and 10 mg/kg, i.v.) also failed to block rhythmic climbing fiber responses. Experiments performed in decerebrate, harmaline-treated rats confirm that diazepam inhibits the peripheral tremor without blocking the rhythmic burst discharges of Purkinje cells.

These results lead us to conclude that diazepam suppresses harmaline tremor by acting at the level of the spinal cord.

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153 CEREBELLAR CORTICONUCLEAR PROJECTION DEMONSTRATED BY THE HORSERADISH PEROXIDASE METHOD. J. Courville and F. Faraco-Cantin*. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec.

Injections of horseradish peroxidase (volumes: 0.2 µl - 0.8 µl; concentrations: 10% - 60%) in the intracerebellar nuclei and in the lateral vestibular nucleus (NVL) label the Purkinje cells which distribute their axons to these nuclei. Following injections in the fastigial nucleus, labelled cells are located within a sagittal band on the same side. In the most rostral lobules, the width of this band is approximately 0.75 mm while it extends for about 1.5 mm in the intermediate lobules. In lobule VII, the band of cells projecting to the fastigial nucleus is wider and in lobule VIII, it extends up to the paramedian sulcus. The projection to NVL originates from a band adjacent to the one just described. Its width is about 0.5 mm in the anterior lobe and 1 mm in intermediate lobules. Cells projecting to the NVL are present in small number in lobules VI to VIII and abundant in the caudalmost lobules. The cells projecting to the interposed nuclei are located within a band measuring about 2.5 mm in the anterior lobe and up to 3.5 mm in lobule VI. In the posterior lobe, cells connected to these nuclei are found in the dorsal part of the folia of Crus II, in the medial regions of the folia of the paramedian lobule and occasionally, in the vermis. Cells projecting to the dentate nucleus are located along the lateral borders of the anterior lobe folia, throughout Crus I and the paraflocculus, in the ventral portions of Crus II and in the lateral parts of the paramedian lobule.

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154 SIMULTANEOUS CEREBELLAR PRESSOR AND DIVE RESPONSES IN THE ANESTHETIZED DOG. K. J. Dormer* and H. L. Stone. Marine Biomedical Institute, Univ. of Tex. Med. Branch, Galveston, TX. 77550.

The objective of this study was to observe the net heart rate changes resulting from combined fastigial pressor (FPR) and nasal-evoked "dive" responses. The FPR in the dog is elicited by stimulation in the rostro-medial portions of the cerebellar fastigial nucleus (FN) and is characterized by increased heart rate, arterial pressure and myocardial contractility (Dormer & Stone, Soc. Neurosci. p. 429, 1975). Reflex bradycardia and variable changes in arterial pressure, similar to cardiovascular adaptations in diving mammals, have been elicited by stimulation of the nasal mucous membrane in dogs by Angell James and Daley (J. Physiol. 220:673-696, 1972). Mongrel dogs were anesthetized with alpha chloralose (110 mg/kg) and the FN was electrically stimulated using concentric electrodes and bipolar stimulation. Tachycardias up to 150 bpm and arterial pressure rises up to 100 mm Hg could be elicited. Next, balloon catheters were inserted into the nostrils and nasopharyngeal meatus through which water or saline could be pumped to stimulate nasal receptors. Flow rates of 300-700 ml/min were sufficient to initiate the reflexes and resulted in bradycardias up to 36 bpm. Blood pressure initially fell then increased during the nasal perfusion. When both the FPR and nasal reflex were simultaneously evoked there was a partial cancellation (15-30%) of the fastigial tachycardia. The bradycardia and tachycardia did not sum algebraically. The nasal-evoked cardiac reflexes are proportedly mediated through the trigeminal nerve to the nucleus tractus solitarius (NTS). Recent anatomical studies have also linked the fastigial nucleus with the NTS. This study suggests a specific inhibition between cardiac cerebellar-sympathetic and trigeminal-parasympathetic neurons in medullary regions. Supported by NIH grant HL 05145.

155 CEREBELLAR ADAPTATION CONTROL IN HUMANS. Robert J. Grimm and Lewis Nashner. Neurological Sciences Institute, Portland, Oregon 97209. Freely standing cerebellar patients (n=15) submitted to a series of brief rotational or translational movements of a platform. Force output from the platform and integrated surface EMG recordings were obtained from four leg muscles, tibialis anterior, quadraceps, biceps femoris, and gastrocnemius. The experiment required that patients use postural reflex controls in a number of different ways: Do cerebellar patients have a long-latency functional stretch reflex (FSR), and if present, can it be appropriately modified as occurs in normal subjects? The FSR was present in all patients with cerebellar deficiencies. Consequently, a blighted cerebellum does not preclude the occurence of an FSR. The principle finding in cerebellar patients was an inability to attenuate or amplify the FSR where appropriate to postural control. Not only do cerebellar patients lose FSR adaptation but there are striking timing errors in the FSR "structuring" (simultaneous occurence of FSR's in muscle linkages to a physiologic stretch). FSR adaptation loss, timing errors in FSR coordination in muscle groups, and increased latency of vestibular-generated responses to falling are the consequences of midline, anterior lobe cerebellar deficits. The clinical effect of these deficits is to produce a stiff-legged, ataxic "stilt walking" gait: each step is a new one. Together with published studies on saccade control and vestibular-occular reflex gain, this is the third example of adaptation losses with cerebellar deficits.

156 MULTIPLE UNITARY ACTIVITY AND MICROSTIMULATION OF THE INFERIOR OLIVE IN RABBITS. D.T. Hess* and N.H. Barmack, (SPON: C.J. Russell). Neurological Sciences Insti., Good Samaritan Hosp. & Med. Cntr., Portland, Or.

We have examined the role of climbing fibers in cerebellar function, by recording multiple unitary activity from the dorsal cap of the inferior olive in anesthetized and unanesthetized rabbits. Cells of the dorsal cap are activated exclusively by visual stimulation of the contralateral eye and are directionally selective to low velocity horizontal motion of the visual field in the posterior-to-anterior direction. The cells have a maximal directional selectivity at 1 deg/sec (50% bandwidth .08-8 deg/sec). The activity of these cells is not modulated by natural vestibular stimulation. After characterizing the trigger features of the dorsal cap neurons, microstimulation (20-50μa 200μsec pulses, 2 sec train) was used to evoke eye movements in unanesthetized rabbits. Microstimulation of the right dorsal cap evoked low velocity (less than 5 deg/sec) conjugate eye movements toward the right, frequently outlasting the stimulus. As a control, a 3mm saggital cut to the left of the midline at the level of the dorsal cap was made; severing the crossed climbing fiber output of the right dorsal cap, but leaving intact its visual input from the ipsilateral pretectum. This cut completely eliminated the eye movements previously evoked by stimulation of the right dorsal cap. The direction and velocity of the conjugate eye movements evoked by microstimulation suggests that climbing fiber activity provides visual feedback to correct the drift of the visual field on the retina. The relative low discharge frequencies of climbing fibers would be adequate to convey this low velocity information. Climbing fibers of the right dorsal cap could evoke eye movements towards the right by disinhibiting the vestibular nuclei indirectly by reducing the net activity of the left flocculus Purkinje cells and/or exciting the left vestibular nuclei directly with axon collaterals. (Supported by PHS Grant EY-00828)

157 THE SYNAPTIC LOCALIZATION OF RUBRAL AND TEGMENTAL AFFERENTS WITHIN THE INFERIOR OLIVARY NUCLEUS. J.S. King and J.A. Andrezik*. Department of Anatomy, The Ohio State University, Columbus, Ohio, 43210.

As part of a continuing effort to describe the synaptic organization of the inferior olivary nucleus, stereotaxic lesions were placed within the red nucleus or the deep tegmentum dorsal to the red nucleus. Injections of horseradish peroxidase and [^{3}H]-leucine reveal that neurons within these areas project to the inferior olivary nucleus (see M. Linauts and G.F. Martin, abstract this meeting).

At survival times of 2 and 3 days degenerating axon terminals are present primarily within the principal olivary nucleus. The rubro-olivary terminals contain spherical agranular synaptic vesicles and typically display a Gray's Type 1 synapse. Their postsynaptic sites are small diameter dendrites or the spiny appendages within the previously described synaptic clusters (King, J.S. et al., 1976, J.C.N. 165:387). Tegmento-olivary axon terminals consistently contain a number of large dense core vesicles (average of 6 per ending) and spherical agranular synaptic vesicles. Degenerating terminals of tegmental origin primarily synapse on spiny appendages (65%) within the synaptic clusters. The remaining terminals (35%) contact the shafts of small diameter dendrites. These results establish that in addition to the previously described cerebello-olivary axon terminals (King, J.S. et al., 1976, Exp. Br. Res., in press), a second major afferent system of mesencephalic origin influences synaptic events within the principal olivary nucleus via the synaptic clusters. Supported by U.S.P.H.S. Grant NS-08798.

158 EFFECTS OF THYROID STATE ON THE CELL CYCLE AND MITOSIS IN DEVELOPING RAT CEREBELLAR CORTX. Jean M. Lauder. Dept. Biobehav. Sci. Univ.Conn.,Storrs.

The effects of thyroid state on the rates of cell acquisition and cell proliferation were studied in the external granular layer (EGL)at 10 days using quantitative autoradiographic methods.

Hyperthyroidism shortens the cell cycle by decreasing the length of G_1, indicating that excess thyroxine exerts a direct effect on the EGL to accelerate the onset of neuronal differentiation (Nicholson and Altman Br. Res. 44: 13-23,1972). Such cells do not leave the EGL prematurely, however, resulting in a decreased labelling index (LI), mitotic index(MI), and growth fraction (GF), as well as an increased doubling time (DT) as non-dividing cells dilute the proliferating cell population.

Hypothyroidism does not appear to change the length of the cell cycle, but like hyperthyroidism results in a reduced LI,GF and increased DT. The MI, however, is not significantly changed, although an increased number of anaphase-telophase mitotic figures are found in the subproliferative zone. This,combined with the observation of an increased number of labeled mitoses with time after isotope injection,indicates that hypothyroid EGL cells may spend a prolonged time in mitosis, an effect masked in the cell cycle analysis and MI, both of which are based on the observed number of mitoses. These cells apparently pass to the subproliferative zone while still in mitosis, then again begin to proliferate as evidenced by an increased LI in this zone where normally little cell proliferation occurs.This prolongation of mitosis explains the reduced rate of cell acquisition in the hypothyroid EGL at 10 days.

These results suggest that proper levels of thyroid hormone are necessary for normal cell proliferation and cell acquisition in the rat cerebellar cortex during postnatal development.

159 THE ORGANIZATION OF OLIVARY PROJECTIONS FROM THE THALAMUS AND MIDBRAIN AS REVEALED BY HORSERADISH PEROXIDASE AND AUTORADIOGRAPHIC METHODDS. M. Linauts* and G.F. Martin (Spon: D.L. Clark). Department of Anatomy, The Ohio State University, Columbus, Ohio 43210.

The origin of midbrain-olivary connections was determined by the horseradish peroxidase method in the Virginia opossum. After multiple injections which fill the entire olive, neurons are labelled in the nucleus subparafascicularis of the thalamus, the nucleus of Darkschewitsch, the fields of Forel, the interstitial nucleus of Cajal and adjacent periaqueductal grey, the nucleus linearis, the tegmentum dorsal to the red nucleus, the midbrain tegmentum, certain areas of the superior colliculus, the caudal extreme of the pretectal complex and, to a limited extent, the rostral red nucleus as usually defined. In subsequent experiments ^{3}H-leucine was introduced into the above areas and the olivary targets of their axons determined by autoradiographic methods. The results suggest that neurons within the ventral periaqueductal grey, the tegmentum dorsal to the red nucleus, the red nucleus itself, the interstitial area, the nucleus linearis, the fields of Forel and the subparafascicular region all project in an organized fashion to the principal nucleus of the inferior olive. Some of these same regions also relay heavily to certain portions of the medial accessory complex. Evidence for projections to specific parts of the dorsal accessory nucleus from the fields of Forel, the subparafascicular region and linear nucleus also is present. Tectotegmental areas relay to small zones of the caudal medial accessory olive. The olivary targets of midbrain fibers are interpreted in light of olivocerebellar organization in the opossum as revealed by the horseradish peroxidase technique. Supported by USPHS NS-08798 and NS-07410.

160 TETRODOTOXIN-RESISTANT DENDRITIC SPIKES IN AVIAN PURKINJE CELLS. R. Llinás and R. Hess*. Div. of Neurobiology, Dept. of Physiology & Biophysics, University of Iowa, Oakdale, IA 52319.

Field potential analysis in pigeon cerebellar cortex has shown large negative potentials at midmolecular layer following stimulation of a) the surface of the cortex (Loc) and b) the underlying cerebellar white matter (WM). These extracellular negativities had distinct all-or-nothing components which were especially visible with small variations of the amplitude of the Loc stimulus and did not reverse with depth. Intradendritic recordings from Purkinje cells at midmolecular layer indicated that these negative potentials were generated by prolonged dendritic spikes (5 to 10 msec in duration). As in other species, these spikes appear to originate from several sites in the dendritic tree since progressive hyperpolarization through the recording electrode revealed discrete all-or-none components. At somatic level the prolonged extracellular negative action potentials were modified to the typical positive-negative short-lasting spikes. Following the application of 10^{-5} tetrodotoxin and 5mM 3-aminopyridine, field potentials produced by Loc and WM stimulation were abolished. However, it was still possible to record the large extracellular negativities at the molecular layer. Intradendritic recordings under these conditions showed prolonged multi-notched spikes following direct stimulation through the recording electrode or a strong stimulation of the surface of the cortex in the immediate vicinity of the recording site. Local cortical application of cobalt chloride or manganese chloride abolished this electrical activity. It is thus concluded that the dendrites in avian Purkinje cells are capable of supporting action potentials and that the action currents are probably carried, in part, by calcium ions. Supported by PHS grants NS-09916 and NS-05748 from NINCDS.

161 RETICULO-OLIVARY CONNECTIONS, THEIR ORGANIZATION AND POSSIBLE SIGNIFICANCE. G.F. Martin, M.S. Beattie, H.C. Hughes and M. Linauts. Department of Anatomy, The Ohio State University, Columbus, Ohio, 43210.

Although in a previous report we described the conformation of the opossum inferior olive as well as certain of its connections, it was not possible at that time to detail reticulo-olivary projections because of the techniques employed. However, in light of physiological data implicating the reticular formation as a possible relay through which spinal influences reach the inferior olive, we have undertaken a detailed analysis of reticulo-olivary connections by autoradiographic methods. Such studies reveal that reticulo-olivary fibers arise within the medulla, pons and tecto-tegmental portions of the midbrain and that specific reticular nuclei often have surprisingly limited and precise olivary targets. For example, fibers from the nucleus gigantocellularis distribute to portions of subnucleus "c" of the medial accessory complex, whereas parts of the nearby nucleus pontis centralis caudalis relay to a closely adjacent area of subnucleus "b". In general, however, some portion of the caudal medial accessory nucleus is the major recipient of reticular input. Parallel experiments show that many of the neurons in the olivary targets of the reticular formation are positive for horseradish peroxidase activity after injections into "spinal" areas in the cerebellar cortex (anterior lobe, paramedian lobule and pyramis), although some of them also contain reaction product after injections of other cerebellar cortical areas. In summary, it appears that at least some reticulo-olivary circuits are organized to provide routes through which information gains access to the "spinal" cerebellum. Supported by U.S.P.H.S. Grants NS-07410 and NS-08798.

162 INTRACELLULAR STAINING OF PURKINJE CELLS AND THEIR AXONS WITH HORSERADISH PEROXIDASE. McCrea, R.A., Bishop, G.A., and Kitai, S.T., Wayne State University, School of Medicine, Department of Anatomy, Morin Memorial Laboratory, Detroit, Michigan.

In the course of a study of the organization of the cerebellar cortico-nuclear projection system of the cat, Purkinje cells in the anterior lobe, lobus simplex and the paramedian lobule were stained by filling intracellular recording microelectrodes with a 4% solution of horseradish peroxidase (Sigma Type VI). Following penetration of a Purkinje cell, this solution was ejected with 15-30 nA positive DC pulses 200 msec in duration for 1/2 hour. After survival times of 6-27 hours, the animals were perfused intracardially with a buffered fixative of 1% gluteraldehyde and 3% paraformaldehyde. Frozen sagittal sections were cut at 75-100μ, reacted with 3.3 diaminobenzidene tetrahydrochloride and H_2O_2, mounted and lightly counterstained. The main axon, axon collaterals and dendritic tree of the injected neurons were stained brown or black, depending on the amount of current injected. The axon could be followed for long distances, and in some cases to its terminal arborization in the cerebellar nuclei. Purkinje axons gave off extremely delicate collaterals to the Purkinje cell layer and granular layer near to the soma, which correspond to the supra- and infraganglionic plexae of Cajal. After leaving the folium in which the cell body was located, Purkinje axons typically coursed toward the deep nuclei, pausing occasionally at the mouth of a lobule to loop or double back before resuming a nuclear bound trajectory. In the nuclei, the axons became quite faint, but could be observed to branch repeatedly, ending in many fine beaded terminals. (This work was supported by NIH Grant 00405 and RR 5384.)

163 ANATOMIC EVIDENCE FOR TWO TYPES OF SYNAPTIC INTERACTION AMONG CELLS WITHIN THE BASILAR PONTINE NUCLEI. G.A. Mihailoff. Dept. Cell Bio., Univ. of Tex. Health Sci. Ctr, Southwestern Med. Sch., Dallas, Texas. 75235.

Previous studies of Golgi impregnations of opossum basilar pontine nuclei demonstrated the existence of two classes of neurons, namely projection cells and intrinsic neurons. It seemed reasonable then to look for evidence of synaptic interaction between these cells and this paper will present morphological details supportive of such linkages. Further examination of Golgi material indicates that projection neuron axons give rise to collaterals that course for distances up to 300μm within the pontine gray. Thus, a potential substrate exists for interaction between pontine regions (1) receiving input from different cerebral cortical zones or totally different afferent systems or (2) projecting to different regions of the cerebellar cortex. EM corroboration of this finding was achieved by subjecting a series of animals to cerebellar cortical lesions. This induced a retrograde reaction in basilar pontine neurons which manifested itself in somata, dendrites, axons and in a group of axon terminals. Such reactive terminals were never observed in previous EM studies of control material and can be distinguished from the terminals of the two major afferents, the corticopontine and cerebellopontine axons which were also identified in earlier work. On this basis it is our contention that these terminals arise as collaterals of pontocerebellar axons. Potential synaptic interaction within the pontine gray is further augmented by the discovery of dendrodendritic synapses which, when correlated with previous Golgi observations suggests that such presynaptic dendrites arise as thin beaded processes from intrinsic cell dendrites. Thus, two morphological substrates for synaptic interaction exist within the pontine nuclei and are mediated by intrinsic cells as well as projection neuron recurrent collaterals.

164 THE CYCLIC NUCLEOTIDE SYSTEMS IN THE CEREBELLUM OF THE WEAVER, NERVOUS AND STAGGERER MUTANT MICE. N.S. Nadi* and W.J. McBride. (Spon. V. Milstein). Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN. 46202.

The levels of adenylate cyclase, guanylate cyclase, adenosine 3',5' monophosphate (cyclic AMP), and guanosine 3',5' monophosphate (cyclic GMP) were measured in the cerebella of control and neurologically mutant mice: weaver and staggerer (absence of granule cells), and nervous (absence of Purkinje cells). The activities of synthetic enzymes in the mutant mice were not significantly different from the controls when expressed on the basis of mg protein. The basal levels of cyclic GMP in incubated cerebellar slices of the nervous mutants and the weaver mutants were significantly lower than control values. The cyclic GMP content of the staggerer cerebellar slices was not significantly different from the control values. When stimulated by 121 mM K+, the increase of the cyclic GMP in the slices from the nervous mutant was significantly higher than that of control slices incubated under the same conditions. In the case of the weaver mice, K+ stimulation did not produce results significantly different from the control values. Cyclic AMP levels were significantly higher in incubated slices from the weaver mice than controls, and significantly lower than controls in slices from the nervous mutants. The levels of cyclic AMP in incubated slices from the staggerer cerebellum were not significantly different than control values. The elevation of cyclic AMP caused by exposure to K+ was significantly higher than control values in the slices from weaver mutants, but not significantly different from the control values in the case of the nervous mutants. The observations on the basal levels of the cyclic nucleotides in slices from cerebella of mutant mice appear to indicate that certain neurons in the cerebellum contain large pools of these compounds. (Supported by Grant MH-03225-17 from NIMH).

165 MANGANESE SENSITIVE POTASSIUM RELEASE DURING SURFACE STIMULATION IN CAT CEREBELLUM.
C. Nicholson, R. Steinberg* and G. ten Bruggencate*
Physiol. Inst., Univ. Munich, Fed.Rep. Germany

Field potentials were evoked by surface stimulation in the cat cerebellum, recorded with a 7-micropipette array and electronically processed (Nicholson and Llinás, Brain Res. 100:418, 1975) to yield the current source density (CSD). The center electrode of the array was a double barrelled micropipette, one side of which was a K^+-sensitive ion-selective electrode. CSDs associated with both presynaptic parallel fiber volleys and postsynaptic dendritic activation as well as extracellular K^+ concentration, $[K^+]_o$, were measured during superfusion of the cerebellum with Ringer solution and Ringer plus 2.5 mM $MnCl_2$. Repetitive stimulation (5-20 Hz) under normal Ringer produced increases of several mM in $[K^+]_o$ followed by an undershoot on stimulus cessation. Mn-Ringer reduced the increases in $[K^+]_o$ and abolished the postsynaptic CSD. Mn-Ringer left the parallel fiber CSD unaltered. We conclude that a significant amount of the $[K^+]_o$ increase during repetitive cerebellar stimulation is Mn-sensitive, and hence Ca^{++} related, and correlated with synaptic or dendritic events. Supported by Deutsche Forschungsgemeinschaft grant Br 242/12 and the Alexander von Humboldt Foundation.

166 DISCHARGES OF MONKEY FLOCCULUS UNITS IN EYE MOVEMENTS. Hiroharu Noda, and Reizo Asoh*. Brain Research Institute, Sch. Med., UCLA, Los Angeles, CA. 90024

Simple spike discharges of Purkinje cells (P-cells) were studied in the flocculus of Macaca nemestrina. A typical response of these cells to saccadic eye movement was complete suppression of firing. The majority of P-cells (56 %) ceased firing 10-20 msec prior to the initiation of a saccade (regardless of its direction) and resumed firing as soon as the saccade was completed. In other P-cells (17 %), the suppression occurred when the eyes moved in only one direction and a transient increase in firing (or no change) was observed with a saccade in the opposite direction. The remaining P-cells (27 %) showed only a facilitatory response to saccadic eye movement, and some of these cells were also directionally selective.

During steady eye position,P-cells showed a considerably high level of tonic discharges of simple spikes (60-100 Hz). In half of these (51 %) the level of tonic firing changed as a function of fixation points,and the level of firing became more than twofold when the eyes were in the preferred direction of gaze. Although there was no clear relationship between the firing rate and the eye position in the orbit in some units, discharge rate was clearly a linear function of eye position in the others. This suggests that some P-cells carry information on eye position.

Movement of a pattern in the visual field resulted in a dramatic modification of the firing pattern of some P-cells. These cells were easily identified because this modification was not present when testing in total darkness. It is argued that this modification of simple spike discharges by a moving pattern indicates that the visual information is transferred to the P-cells by mossy fiber inputs (Supported by NIH Grant EY01051).

167 EFFECTS OF LASER IRRADIATION ON THE SPONTANEOUS ELECTRICAL ACTIVITY OF UNSTAINED CEREBELLAR CELLS IN CULTURE. James Olson*, Walter Schimmerling*, Abdel M. Mamoon, and Cornelius A. Tobias*. Donner Laboratory, University of California, Berkeley, CA 94720 and Department of Physiology and Anatomy, University of California, Berkeley, CA 94720.

We report on a continuing study of the effects of laser radiation on cultured Cerebellar Purkinje and Golgi cells. The cells are grown in a plasma clot as explants from the cerebellum of two to four day-old rats. The electrical activity is recorded extracellularly. The distribution of time intervals between the action potentials of these neurons can be well approximated by a combination of gaussian and poisson frequency distributions. We have designed an instrumentation system which incorporates a commercial ruby laser (694.3 nm) which was available for our studies. The laser light is focused to a 4 μm diameter spot on a single unstained neuron. The energy of each laser pulse is measured by means of a calibrated photodiode which receives a small fraction of the total laser output. The laser may be triggered to coincide with an action potential or delayed a fixed time interval after an action potential. We have found that a single pulse of ruby laser radiation is capable of modifying the spontaneous activity of cerebellar neurons. The width of the gaussian component and the mean rate of the poisson component were found to decrease in response to the laser pulses. A threshold in total energy density delivered to the cell (1 $mJ/\mu m^2$) exists for the latter effect if the laser pulses are delayed with respect to an action potential. Cells which receive laser pulses timed to coincide with an action potential do not show such a unique effect. Laser irradiation also caused qualitative changes in the neuron's activity such as short term reversible interruption of electrical activity.

168 SYNAPTOGENESIS IN THE CEREBELLAR CORTEX OF THE SYRIAN HAMSTER, MESOCRICETUS AURATUS. Mary Lou Oster-Granite. Dept. Neurol., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Hamsters aged each day from birth (day 0) through postnatal day 42 were perfused with one of a variety of mixed aldehyde or picrate-aldehyde fixatives. Their cerebella were processed conventionally for electron microscopy. We found scattered parallel fiber and climbing fiber synapses present on immature dendrites of Purkinje cells at birth. Numerous growth cones were found in the developing molecular layer during the first six postnatal days. At day 6, basket cell axosomatic and axodendritic synapses onto Purkinje cells were present, at a time when the Purkinje cells showed little cytologic differentiation and still possessed perisomatic spines. Immature, almost smooth, climbing fiber and mossy fiber rosettes were present as early as day 8. These rosettes already made synapse with Golgi and granule cell dendrites in the developing internal granular layer. Golgi axons contacted both Golgi and granule cell dendrites in the immature glomeruli by day 13. By day 16, the cortex appeared mature with glial processes investing most synaptic and nonsynaptic structures. By day 21, no external germinal layer remained, and the processes and synapses continued to mature through postnatal day 42. Based on comparisons with the developing cerebellum of the rat and mouse, the newborn hamster cerebellum is more immature at birth, and thus may provide a good experimental system for early studies of cerebellar recognition mechanisms which lead to differentiation and synaptogenesis.

169 EFFECTS OF ELECTRICAL STIMULATION OF THE CEREBELLUM OF VENTILATION AND CIRCULATION IN THE CAT. John W. Patrickson, C. O. Trouth, James A. Holloway, Rita Brooks. Dept. of Physiology Coll., Med., Howard University, Washington, D.C.,20059

The cerebellum of 18 cats anesthetized with chloralose-urethane (40 mg/kg clucochloralose, 200 mg/kg urethane) was exposed following the removal of the supraoccipital portion of the occipital bone and systematically explored, at millimeter intervals in three coordinates, by electrical stimulation (rectangular impulses 40/sec; 1msec; 4-6 volts) with unipolar Tungsten electrodes (tip diam. 3-5μm). Circulatory and respiratory responses were monitored and mapped into cerebellar sections. Blood pressure responses were almost exclusively pressor, vasodepressor responses being seldom observed. Pressor responses were concentrated around the anterioventral portion spreading laterally from the midline. Ventilatory responses were more extensive than vasomotor responses and in many instances both were elicited from the same points.

Inspiratory effects were concentrated in the anterioventral portion extending laterally from midline and including the central nuclei while expiratory responses were elicited from a small field in the lateral portion. (Supported by NSF Grant HES 75-09024 and NIH Grant 1 TO 2 GM 05010-01).

170 SOURCE OF CEREBELLAR CLIMBING FIBERS IN TURTLES.
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Most investigators consider the mammalian inferior olive (IO) as the source of cerebellar climbing fibers. A homologue for this nucleus is unknown for reptiles. A corresponding cell group has been searched in turtles (Chrysemis geographica, Graphthemys scripta elegans). Injection of horse radish peroxidase into the cerebellar cortex resulted in perikaryon labelling within a variety of cell groups including dorsal collumn -, reticular -, and vestibular nuclei. When the injection was restricted to the molecular layer labelled neurons were only found within one cell group located in the alar plate just rostral to the vestibular complex. Cells within this nucleus can be antidromically invaded by electric shocks applied to the molecular layer via bipolar electrodes. Electrolytic lesions in this nucleus greatly reduced climbing fiber (CF) field potentials in response to limb nerve stimuli within the corpus cerebelli. Cerebellar surface evoked CF potentials in response to brain stem stimuli were of shortest latencies when the stimulation electrode was located in the alar plate rostral to the vestibular nuclei. These data suggest that the turtle's climbing fibers originate from a cell group adjacent to the vestibular complex which may correspond to the location of mammalian embryonic inferior olivary cells in the alar plate prior to their migration towards their ventral destination.

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171 SOMATOTOPIC ORGANIZATION OF RAT CEREBELLAR TACTILE CORTEX USING MICRO-MAPPING METHODS.
Georgia M. Shambes, John M. Gibson and Wally Welker.
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Little is known about the detailed pattern of organization of somatosensory afferent circuits to cerebellar cortex. We mapped the tactile projections from body surfaces to Crura I and II and the paramedian lobule of the albino rat's cerebellum. Activity of multiple units in the granule cell layer evoked by gentle mechanical stimulation was recorded by tungsten-ball microelectrodes (3 μ diameter) in rats anesthetized with sodium pentobarbital. Data were obtained from about 700 recording locations. As many as 50 microelectrode punctures were placed within each mm^2 of cortical surface. 1. The tactile area was primarily confined to the medial aspects of Crura I and II and the adjacent intermediate zone (folial crowns, buried folial surfaces), exclusive of vermis. 2. Stimuli optimal for activating granule cell unit activity were exclusively tactile; units being highly responsive to extremely light deformation of hair, glabrous skin, or dental contact. 3. Projections were predominantly ipsilateral and from the head, especially perioral and intraoral hairy skin, incisors and facial vibrissae. Bilateral and contralateral projections were situated successively more medial in the intermediate zone. 4. Tactile projections were organized somatotopically in a distorted variegated pattern, but with larger receptive fields than found for rat cerebral SI cortex (C. Welker, 1971). 5. Perioral projections were situated in Crus II and facial and head projections in Crus I and its buried gyrus. 6. Projections from different adjacent peripheral regions were oriented in adjacent mosaic patches with overlapping edges in each folium. This is the first study to delineate fine details of somatotopic organization of tactile projections to cerebellar cortex utilizing micromapping recording methods. (Supported by USPHS Grants NS6225 and NS07026.)

172 EVIDENCE FOR THE BRANCHING OF CEREBELLAR EFFERENT AXONS. D.L. Tolbert, H. Bantli, and J.R. Bloedel. Depts. Neurosurg. and Physiol. U. of Minn. Minneapolis, Mn. 55455.

Neuroanatomical and electrophysiological experiments were performed in cats to test the hypothesis that axons from neurons in the dentate (DN) and interposed (IN) nuclei branch and project to different areas in the brainstem. Following injections of horseradish peroxidase (HRP) into the contralateral thalamic ventrolateral nucleus (VL), small (10-15), medium (19-24), and large (29-35) neurons in the DN and IN were observed to be HRP-positive. Only small neurons were labeled following HRP injections in the contralateral inferior olive (IO), while the medium-sized neurons were labeled following injections restricted to the cerebellar cortex. These anatomical findings indirectly suggest that the small neurons in the DN and IN project to both the VL and IO and that the medium-sized neurons project to both the VL and the cerebellar cortex. Since previous electrophysiological experiments have demonstrated that the cerebellar nucleocortical fibers are likely collaterals of cerebellar efferent fibers, a second series of studies was undertaken to directly demonstrate that axons of neurons in the DN and IN project to both the VL and IO. The results showed that numerous neurons responded antidromically to stimulation of both the VL and IO. Eight units were completely analyzed by performing collision experiments between the antidromic responses evoked by both the VL and IO stimuli. These experiments indicated that the shortest interval at which collision occurred was the same independent of which stimulus was applied first. Since this interval was considerably longer than the measured refractory period, the two antidromic responses were likely evoked in the same neuron by activating separate branches of its axon. This work was supported by NIH Grant NS-09447-05 and NIH Contract NO1-NS-2332.

173 ANALYSIS OF CEREBELLAR INTENTION TREMOR IN THE MONKEY. T. Vilis and J. Hore. Department of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1.

Oscillations of the forearm, resembling intention tremor seen in patients with cerebellar lesions, were produced in Cebus monkeys using the technique of reversible cooling of the dentate and interpositus nuclei. By training the animals to rotate a handle in a horizontal arc, movements were largely restricted to the elbow joint which resulted in oscillations that were sinusoidal in nature. These oscillations were especially evident following fast, step tracking, movements and following perturbations to the arm which the animal was taught to resist. Changes in the periphery altered the character of the tremor. For example an increase in the inertia of the handle decreased both the frequency and amplitude of the oscillations. Perturbations applied during on-going oscillations resynchronized the phase of the oscillations as monitored in the movement, the muscle activity, and single unit activity in the precentral cortex.

The character of the tremor was also altered by varying the degree of dysfunction of the dentate and interpositus nuclei. By grading the intensity of cerebellar cooling, the frequency of oscillations could be altered in a continuous fashion from 6-8 Hz at 39°C to 3-4 Hz at 2.5°C. In addition, the nature of the oscillations following both movements and perturbations became progressively less stable and of a larger amplitude the greater the cooling.

Our results suggest that at least two factors determine the nature of cerebellar intention tremor: peripheral input and the degree of cerebellar dysfunction.

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174 CEREBELLAR UNIT ACTIVITY IN THE AWAKE AND UNANESTHETIZED CAT DURING SPONTANEOUS EYE MOVEMENTS. B.D. Waterhouse*, L. Mays and J.G. McElligott. Dept. of Pharm., Temple Univ. Medical School, Philadelphia, Pa. 19140

Single unit activity from vermal lobes VI and VII was recorded in awake, unanesthetized cats during periods of spontaneous eye movements in the light and dark. Bilateral neck EMG activity (splenius m.) was recorded and auditory (buzzer) and visual (light flash) stimuli presented as controls since these forms of sensory input are known to project to this area of cerebellum. During recording sessions data was stored on magnetic tape and later analyzed using a PDP-11 computer programmed to examine the relationship of spike train activity to direction, and onset or cessation of eye movement. A total of 162 neurons were observed and eighty-six of these comprehensively analyzed. Twenty cells (23%) demonstrated spike train activity changes in a temporal relation to the onset of saccadic eye movements. All eye movement related (EMR) neurons maintained tonic firing levels (avg.=55 spikes/sec) upon which were superimposed short increases or decreases in activity related to eye movements. Changes in EMR neuron activity were usually monophasic, either increase or decrease in firing rate (16 cells); but four cells demonstrated biphasic activity (increase/decrease combination of changes). Monophasic cell activity changes occurred either before (20 $\pm$ 10ms), or after (22 $\pm$ 13ms) the onset of the particular movement with the duration of activity change ranging from 20ms to 175ms. EMR cells were also directionally specific demonstrating consistent activity changes for right (35%), left (25%) or both (40%) directions of horizontal movement. Seven EMR neurons were identified as Purkinje cells, based on the presence of the climbing fiber response. Climbing fiber responses were not related to any eye movement parameter examined. Supported by NINCDS grant No. 10488.

Cerebral Cortex

175 LOCATING CORTICOSPINAL NEURONS IN MATURE, INFANT AND MALFORMED (RADIATION) RATS WITH RETROGRADE AXONAL TRANSPORT OF HORSERADISH PEROXIDASE.
S.P. Hicks, and C.J. D'Amato. Department of Pathology, University of Michigan, Ann Arbor, Michigan 48109.

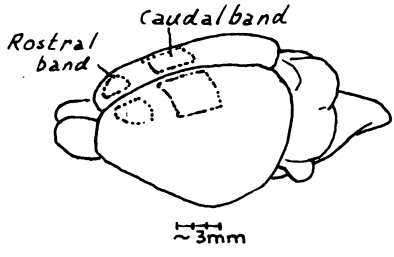
HRP crystals introduced into the site of a partial transection of the rat's spinal cord, which included the corticospinal (CS) tracts, selectively labeled CS neurons. These cells were pyramids in layer V of the motor-sensory cortex; they formed two dorsomedial bands separated by a gap: a small one rostrally and a more extensive one caudally. The latter was about 4 to 4.5 mm long and 2.5 to 3 mm wide corresponding to the hind limb, trunk, and most of the forelimb "motor" region of Hall's and Lindholm's (1974) somatotopic maps. The medial border was 0.5 mm from the midline, and the longitudinal extent was about 4 to 8.5 mm rostral to reference frontal plane 0 in Konig and Klippel (1963). Labeling from the 1st or 7th cervical (C1,7) level marked cells throughout both bands. Labeling from the 1st lumbar (L1) level however revealed cells evenly distributed throughout the caudal band — not just in the hind limb somatotopic region.

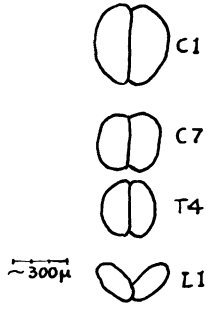
Semiquantitative estimates were made of the numbers of CS cells projecting to the cord. The ratio of the cross-section areas of the CS tracts in the spinal cord at C1, C7, T4 and L1 levels were 5:3.5 :3:1. Assuming cross sections were proportional to the number of axons and using Bernstein's (1966) figure of about 6000 axons in each tract at the thoracic level, there were estimated to be about 10,000 axons at C1 and consequently 10,000 CS cells in each cortex projecting as far as the cervical cord. The number of labeled cells varied among animals, but highest counts based on samples from serial sections (corrected, Abercrombie, 1946) gave estimates of 6500 cells labeled from C1, 2900 from C7, and 1400 from L1, indicating that around half of the CS cells could be marked.

The distribution of CS cells in infant rats labeled at 10 days of age resembled that of the mature, except that there was no gap. How the gap ultimately formed is not yet known.

Among the reproducible malformations of the cortex that can be induced in rats by prenatal irradiation, one of the most disorderly patterns is that following 200R on the 17th day of fetal life, and the affected animals at maturity have severe locomotor deficits. In such animals HRP labeling revealed that some CS neurons had developed in what corresponded to motor-sensory cortex, although they were frequently ectopic and sometimes inverted.

The diagrams schematize the bands of normal cells located by HRP, and the cross section areas of the CS tracts in the spinal cord.
(USPHS NS 10531)





176 EFFERENT DISTRIBUTION FROM THE CORTICAL GUSTATORY AREA IN RATS. R. Norgren and H. J. Grill. The Rockefeller University, New York, N. Y. 10021.

The cortical gustatory area (CGA) was visualized by a lateral approach. A point 1.0 mm dorsal and 0.5-1.0 mm anterior to the intersection of the middle cerebral artery and the rhinal fissure (RF) approximates the center of CGA (Norgren & Wolf, Brain Res. 92: 125, 1975). Tritiated proline or leucine (50 μCi/μl; ≈20-700 nl) was injected at these coordinates (5 rats) as well an anterior, posterior, dorsal, and ventral to them (5 rats). After 48 hrs., the brains were sectioned, prepared for autoradiography, and exposed for 2-8 weeks. Most injection sites were large with respect to CGA, so the projections of gustatory cortex must be inferred from comparison with control injections. Within the cortex, efferents cross to contralateral suprarhinal cortex at the injection site level, and ipsilaterally penetrate deep layers of cortex posterior to CGA. This intracortical distribution extends just ventral to RF and caudally into entorhinal cortex. Fibers exit cortex as discrete fascicles across ventral caudate which is filled with label. In the amygdala a weak, but consistent, distribution occurs in the central nucleus. The far-lateral hypothalamus also contains label, but it appears to originate from sulcal cortex anterior to the gustatory area. The major fiber bundles leave globus pallidus in two groups -- one entering the internal capsule, the other filling the thalamic reticular nucleus and passing into the ventrobasal complex via the external medullary lamina. By far the densest distribution accumulates in the medial third of the thalamic ventrobasal complex -- the lingual and trigeminal relay zones. In most cases this distribution continues into midline and posterior nuclei, but less densely. The midline thalamic projection may not originate in CGA, since it is stronger after injections centered anterior in cortex. Labelled fibers leave the pyramidal tract throughout midbrain, pons, and medulla. Some distribute in substantia nigra, others contribute to a weak, largely ipsilateral distribution in the parabrachial nuclei. Most cross through the reticular formation (RF) and end in a distribution of label which envelops the nucleus of the solitary tract and subjacent RF. This projection is primarily contralateral. A small contingent of labelled fibers continues caudally in the pyramidal tract to the contralateral cervical spinal cord. To one degree or another, the cortical gustatory area feeds back on each of the central afferent relays for taste, and in this respect is similar to the remainder of sensory cortex. (Supported by NIH Grants NS 10150, AM 02360, and GM 1789.)

177 LIFE-SPAN CHANGES IN SHORT-TERM MEMORY IN RELATION TO CELL LOSS IN THE CORTEX OF THE RAT DETERMINED BY AN AUTOMATED, QUANTITATIVE IMAGE-ANALYZING SYSTEM. J.M. Ordy, K.R. Brizzee and W.J. Hansche.* Delta Primate Center, Covington, La. 70433, Tulane Medical School and Psychology Department, Tulane University, New Orleans, Louisiana.

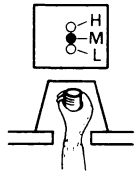
Experiments with human and animal subjects have indicated: 1) declines in short-term memory and 2) loss of cells in some regions of the brain during aging. However, age declines in short-term memory are confounded with general declines in performance. Age declines in short-term memory retention may be related to reduced learning capacity, altered motivation or arousal, loss of motor coordination or some combination of these factors as determinants of age declines in performance generally observed in senescence. Cell loss from the brain has been estimated by descriptive, non-automated methods. The aims of this multivariate research program have been to examine life-span changes in performance with particular emphasis on short-term memory in relation to cell packing density in the cerebral cortex of the rat. According to survival data from a sample of 572 male Fisher 344 rats, the median or 50% mortality occurred at 659 and the longest or maximum survival extended to 1192 days in this strain (Chesky, J. and Rockstein, M., 1975, The Gerontologist, 15(5):29).

The specific aims of this study were as follows: 1) Compare the behavior of 5 young (11 months), 5 adult (17 months) and 5 old (29 months) Fisher male rats on the following tests: (a) open field exploration, (b) auditory habituation-dishabituation, (c) passive-avoidance learning, (d) 2 and 6 hour short-term memory or retention, and (e) pain-elicited aggression; 2) Examine age differences in brain weight; 3) Compare age differences in cortical depth of visual area 17 and auditory area 41; 4) Examine with a computer-programmed automated cell counting and image analyzing system (Leitz TAS) age differences in cell packing density in auditory area 41 and visual area 17; 5). Perform a statistical multiple regression analysis to identify significant relationships between age and all 34 behavioral and morphological dependent variables.

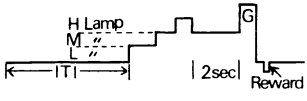
Statistically non-significant performance declines were observed in the old rats in some measures of open field exploration, pain-elicited aggression and auditory habituation. The young group required significantly fewer days to criterion in passive-avoidance learning. The old group of rats was significantly inferior in 6-hour short-term memory retention. Age differences in brain weight were not significant whereas cortical depth in areas 17 and 41 decreased significantly in the old group. Significant decreases in neuron packing density were observed in 17 but not in area 41. Criteria for differentiating small neurons from glia, endothelial cells and pericytes were incomplete for the automated image-analyzing evaluations. Since primary interest was focused on the relationship among days to criterion of passive-avoidance learning, short-term 6-hour memory retention and neuron packing density in area 17 with age, a multiple regression analysis was performed. The 3 variables correlated -0.73, -0.56 and 0.30 respectively with age. The correlation of age with the 3 variables combined was R=0.86. In the combined R of 0.86, the highest correlation of age was with area 17 neuron packing density r=0.73, with 6-hour passive-avoidance memory retention the increase was to R=0.80, and with days to criterion learning the increase was to R=0.86. According to the regression equation for the prediction of age from the 3 variables, no errors of prediction would be made if the predicted ages were used to indicate group membership in the young, mature or old age groups. From the univariate and multivariate evaluations, it was concluded that: 1) age declines in short-term memory were related to reduced passive-avoidance learning in the presence of non-significant age declines in performance; and 2) that the age declines in learning and short-term memory of old rats were also correlated with age differences in neuron packing density in the cerebral cortex. (Supported in part by NIH Grants HD/NS 09942 and RR00164-14).

178 SINGLE UNIT ACTIVITY IN THE DORSOMEDIAL PARTS OF PREFRONTAL AND PREMOTOR REGIONS IN THE VISUALLY CONDITIONED MOTOR TASK IN MONKEYS

M. Sakai* (SPON: T. J. Tobias). Dept. Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi, 484, Japan.



Although single unit activities in the dorsolateral part of the prefrontal cortex have been studied extensively in various paradigms using visual cues. Its dorsomedial part has been escaping detailed studies. This study was intended to uderstand the role of the dorsomedial frontal cortex, adjacent to the motor cortex, in a visually conditioned motor task.



Single unit activity was searched in a border area between areas 9 and 6 of Broadmann, while a monkey was performing the following task. In front of the monkey, there was a vertical bar with strain gage and a movable panel with three small cue lamps (red and green LEDS). One of lamps (red) was lit according to the gripping force generated by monkey's hand. From these, upper, middle, and lower lamps, the monkey knew if the produced force was higer, appropriate, or lower. The task sequence was composed of 4 phases. After an intertrial interval (5 sec ITI), the lower lamp was on (start cue phase), signalling the monkey to grip the bar isometrically. If the force 1000-1500gr was maintained for 2 sec (gripping phase), then the color of middle lamp was changed to green (G in the figure). After this signal, he had to relax the force within 0.5 sec (relaxing phase) to obtain a fruit juice. In addition to this task, unit activity was observed to see the response to the visual stimuli. Cue lamps were presented while his both hands were restrained and he was watching lamps quietly with no movements (visual effect test). In this test, temporal relations of ITI, start cue, gripping and relaxing phases were controlled by an experimenter, mimicking the above visual motor task.

Eighty task related units were collected from the area of A28-16, LO-4 and were classified into 4 types; type A units increased the discharge rated during cue phase and decreased during gripping phase; type B units were active only during gripping phase; type C units decreased their rates during gripping phase; and finally type D units began to descharge in the later phase of the ITI and their increased rates continued, through cue and gripping phases until the relaxing phase. The visual effect was tested in 50% of units sampled. All tested units changed their rates responding to cue lamps without accompanied movement. Their response patterns were essentially the same as during motor task. Moreover, above response patterns were also the same even if the panel was moved by 45° toward left or right side, or rotated by 90°. If trials were repeated for 20-30 times without reward, responses to the middle lamp and green lamp disappeared gradually. If, after this extinction, juice was given, the response patter of B and D units returned quickly after 1-2 rewarded trials. These additional tests indicates that response patterns are produced by visual cue stimuli, free from mechanisms responsible for the isometric flexion and later extension.

These units were predominantly located in the medial aspect of the cortex anterior to A28-A17. On the other hand units in the lateral aspect did not show clear time-locked activity to the events. Intracortical stimulation (< 1.3 mA, 0.3 msec duration, 300Hz) failed to elicit movements in the area of A28-A21 although the motor cortex (A19-)just posterior to this region induced discrete hindlimb muscle contraction (> 0.4 mA).

From these, it may be said that in the medial part of the prefrontal and premotor regions, unit activity, modulated by visual cue and reward may be related to the anticipation and visual attention, but are not directly related to an initiation or maintaining of the voluntary hand movement.

179 GLIAL DEPOLARIZATION AND HYPERPOLARIZATION EVOKED BY INJECTION OF Cl^- IN CORTICAL GLIA. A. Seregin* and R.G. Grossman. Division of Neurosurgery, Univ. of Texas Medical Branch, Galveston, TX. 77550.

The Cl^- permeability and content of mammalian neuroglia have been investigated by intracellular injection of Cl^- in electrophysiologically identified glia of the cat motor cortex. Intracellular recording and ion injection was carried out in 70 glia and 21 neurons. The glia were characterized by MP ranging from -90 to -70 mV, the presence of slow depolarization during electrocortical spindle-bursts, and an absence of spike and synaptic potentials. Cl^- was injected with current pulses of 0.1 to 1 x 10^{-8} A and 100 msec duration, calculated to inject 0.1 to 1 x 10^{-14} M of Cl^-. The initial Cl^- injection, with a single 100 msec pulse, produced glial depolarization of up to 15 mV, which returned to resting levels with a smooth course in 1 to 3 sec. Depolarization was followed by hyperpolarization that attained an amplitude of 3 to 5 mV, and had a duration of 5 to 15 sec, in the cases with the largest preceding depolarization. Subsequent injections of Cl^- resulted in a reduced amplitude of depolarization in response to each pulse, a failure of the MP to return to the pre-injection level, and failure of the hyperpolarizing phase. Hyperpolarization was also abolished when the cortex was cooled to 30° C, or when it was swollen after repeated micropunctures. Cl^- injection in neurons produced transient cessation of spontaneous discharge. The effects of Cl^- injection in glia differ from the effects of Na^+ and K^+ injections. Na^+ injection evoked glial depolarization, high frequency discharge of adjacent neurons and an increase in tissue pulsation mediated by the arterial pulse. K^+ injection had no effect on MP, adjacent neuronal discharge or vascular pulsation. The majority of glia penetrated in these studies are presumed to be astrocytes and their somatic volume can be calculated to be approximately 3 x 10^{-13} l, using a glial diameter of 8 μM (Grossman and Rosman, Brain Res. 28:181, 1971). Injection of 1 x 10^{-14} M of Cl^- can be calculated to produce an initial increase in Cl^- concentration of approximately 33 mM in the glial soma. Since the glial membrane is highly permeable to K^+, injection of this amount of Cl^- into glia should be followed very rapidly by entry of K^+ to maintain electrical neutrality and by H_2O to maintain osmolality, and by significant cell swelling. The observed amplitude and duration of Cl^- induced depolarization and hyperpolarization suggest that glial MP may be partially dependent upon the intracellular concentration of Cl^- and that transport mechanisms for Cl^- may be present.

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180 THE RESPONSE OF TURTLE CORTEX TO REMOVAL OF THALAMIC AND/OR BRAINSTEM AFFERENTS. Leslie M. Smith, Charles C. Ouimet, and Ford F. Ebner. Neurosciences Section, Brown University, Providence, R.I. 02912

The ultrastructural changes in turtle (Pseudemys sp.) visual cortex were studied at various times following either removal of the thalamus on one side, hemisection of the midbrain at midtectal level, or both simultaneously. Animals with survival periods of 7, 14, 21, 28, 40, 50, and 60 days after each type of lesion were perfused with aldehyde fixative in phosphate buffer (details in Ebner and Colonnier, J.C.N., 160:51,'75). Following routine tissue processing, sections from visual cortex were studied for qualitative changes, and selected features of the altered neuropil were quantified.

At 14 days following thalamectomy numerous electron dense vesicle-containing profiles (vcp's) are found within 100um of the cortical (pial) surface. These profiles contain round vesicles and form asymmetrical contacts, usually on large dendritic spines. Degeneration at this stage includes glial wrapping of terminals, ocassionally including an attached post synaptic element. After 40 days electron dense debris is rare in glial cells. The remaining neuropil shows a marked increase in flat vcp's and flat symmetrical contacts, mainly in the superficial part of the molecular layer. There is no apparent change in the density of vacuolar invaginations (VIs) or in small (around 0.5um) tubule-containing profiles. Intermediate degrees of change are present at intermediate times.

At 14 days following midbrain hemisection a few electron dense terminals are seen in the superficial half of the molecular layer. Considerable variability is seen in the shape of the vesicles contained in the degenerating terminals, but they are mainly round vesicles with clear centers and scattered dense cored vesicles of a slightly larger diameter (700A). The few contacts seen have been asymmetrical. The main change at 40 days is a striking increase in small tubule-containing profiles with the highest density immediately beneath the pial surface. Some of these small elements contain vesicles and are presumed axons. Others have been seen in continuity with dendrites. There are changes in the density of VI's over time, with an increase in number of VI's at 40 days; these are frequently seen arising from vcp's that make usual synaptic contacts on the same dendrite.

The combined lesions show more alterations than the sum of both lesions. In thick sections, the neuronal perikarya appear clumped. The sprouting at 40 days is marked, as with the midbrain lesion alone, but normal VI's are not present in the subpial zone and are reduced in number elsewhere.

These results indicate that the reactions produced in cortex due to the removal of thalamocortical inputs differ from those produced by loss of lower brainstem afferents. (Supported by PHS grant no. NS 06551)

181 ORBITOFRONTAL PROJECTIONS TO THE MEDIODORSAL THALAMIC NUCLEUS IN THE DOG.
Duke Tanaka, Jr. Dept. Anat., Col. Med., Howard Univ., Washington, D.C. 20059

A number of studies have investigated the behavioral deficits seen following selective lesions of the orbitofrontal cortex in dogs. The results suggest that this cortical region can be divided into lateral and medial sectors, with each mediating specific functions. Similarities between these results and those obtained following selective lesions of the primate prefrontal region have led several investigators to postulate homologies between the canine lateral and medial orbitofrontal sectors and the primate dorsolateral and orbital prefrontal sectors, respectively. The orbitofrontal and prefrontal regions are both intimately related to the mediodorsal thalamic nucleus (MD). In primates, the ventrally located orbital prefrontal sector projects selectively to the medial MD division while the dorsolateral sector projects to the lateral division. This study was undertaken to compare the corticothalamic projections of the lateral and medial orbitofrontal sectors in the dog and to determine if functionally similar cortical areas in the dog and monkey project to corresponding divisions of MD.

Lesions were made in 17 adult mongrel dogs (8 to 13.5 kg) by subpial aspiration of the lateral orbitofrontal sector (dorsolateral proreal, orbital, and subproreal gyri) and the medial orbitofrontal sector (dorsomedial proreal, medial precruciate, and pregenual gyri). Following survival periods of 5-11 days, the dogs were perfused with 10% formal-saline and the brains processed according to a modification of the Fink-Heimer II silver method.

Lesions of the dorsolateral and dorsomedial proreal gyri as well as those of the medial precruciate gyrus resulted in fiber and preterminal degeneration located primarily within the lateral division of MD. As the lesion was placed more caudally along the medial surface from the proreal to precruciate gyri, the focus of degeneration in lateral MD progressed from a rostrodorsal to a more caudoventral location. Lesions placed more ventrally along the lateral surface (orbital gyrus) resulted in a gradual shift of degeneration more medially within MD, while lesions of the ventrally located subproreal gyrus resulted in dense degeneration within the medial division of MD. Lesions placed ventrally along the medial surface (including the pregenual and medial part of the subproreal gyrus) also yielded degeneration in the medial division of MD.

The results of this study indicate that the dorsal and ventral orbitofrontal sectors project to the lateral and medial divisions of MD, respectively and that the gyri comprising the lateral and medial sectors project to one or the other division depending on their dorsal-ventral location. Additional support for the existence of dorsal and ventral sectors was found in that ventrolateral or ventromedial lesions involving the orbital or subproreal gyri resulted in dense fine-grain degeneration in the ventral medial thalamic nucleus, whereas lesions placed dorsolaterally or dorsomedially did not. The data also indicate that the canine medial and primate orbital cortical sectors interpreted as being functionally homologous do not have corresponding anatomical projections to the mediodorsal thalamic nucleus.

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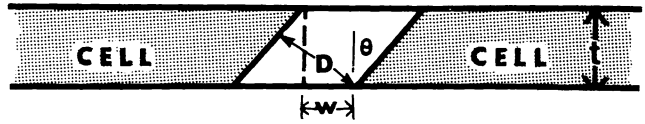
182 AN EFFECT ON VISUALLY DIRECTED BEHAVIOR AFTER TEMPORAL ABLATION.
A.W. Toga*, D.G. Davenport* and S. Horenstein. Saint Louis University, Saint Louis, Missouri 63104.

Modification of a visually dependent learned task following ablation of nonvisual temporal cortex occurred in a series of female cats selected for their ability to be trained in a specially designed equiangular "Y" maze. The maze is equipped for various types of stimulation. Observation is indirect using an overhead mirror. In one set of experiments food was delivered from a feeding cup at the end of each arm of the maze. Animals trained to feed in this manner demonstrated no change in lateral preference after subpial removal of the temporal cortex. It was concluded that any alteration in subsequent behavior would not represent a postural artefact. Appetitive conditioning contingent upon distant light stimulation proved to be difficult for the animals as relatively few achieved criterion. An alternative procedure established visual discrimination as escape from shock delivered to the paws from the electrified floor of the maze. In this procedure the animals were divided into two groups. One was required to avoid or escape in any direction upon any visual stimulus. The other had to move in the direction of a single light when only one was shown or in either direction when two were shown simultaneously. The response to such bilateral simultaneous visual flux (DSS) indicated that each animal established its own pattern of lateralization, though the response to unilateral stimulation was usually appropriate to the side stimulated. After training, subpial resection of the ectosylvian temporal cortex (A_1,A_{11},Epd,Epv) was performed and the animals tested from the 1st to the 10th postoperative day after which they were killed by formol-saline infusion and the brains removed for anatomic study. The side for ablation was chosen opposite that of preference in the belief that any resulting change would be easier to measure. The lesions imposed no postural bias on the control animals. The others trained to discriminate displayed a weak but statistically significant effect on visually dependent avoidance or escape. Contrary to expectation, however, the modification induced was not predictable in terms of the directional effect on the pre-operative bias in response to DSS. That the temporal cortex might influence visually directed behavior might be inferred from the known projections of the ectosylvian region to parts of the adjacent suprasylvian region, to the homologous contralateral cortex in a spatial pattern conforming to the ablation, to both the ipsilateral and contralateral superior and inferior colliculi, and to the medial geniculate body in both spatially determined (A_1) and more diffusely organized (A_{11}, Epv and Epd) patterns. Very large lesions involving the temporal cortex produced clinically obvious neglect of the side of space opposite the lesion and circling toward the latter. In this study the areas ablated were more restricted and the methods of assessment quantitative. The results disclose that lesions of "nonvisual cortex" influence response to visual flux on DSS, but that the effect on direction of choice is not predictable. The former is readily understood in terms of the anatomic projections. Lesions might simultaneously reduce information of presumably facilitatory nature to numerous ipsilateral brain regions including that from layer III pyramidal cells to the opposite cortex where it possibly enters into regulation of excitability of that hemisphere. Thus, a convergent sensory system loses some relatively weak afferent channels at the same time that its contralateral "competitor" is disinhibited.The reason for the second finding is, however, less certain and neither this study nor existing literature are helpful in explaining it. Several possibilities exist including other unidentified modifying influences lost after temporal ablation and unrecognized behavioral differences among individual cats allowing destruction of a weak collateral afferent channel to the visual association cortex to permit the release of alternate mechanisms which govern choice behavior on DSS.

183 MORPHOMETRY OF EXTRACELLULAR SPACE AND DENDRITIC SPINES IN FROG CORTEX. J. Trubatch, A. Loud*, A. VanHarreveld. New York Medical College, Valhalla, N. Y. 10595.

The techniques of quantitative stereology were applied to electron micrographs obtained from frog brain that was stimulated with KCl and then subjected to rapid freeze fixation (1). Morphometric measurements demonstrated that the post-synaptic elements (dentritic spine) occupied 4.84% of the tissue volume in the control experiments and 14.2% in the stimulated preparations, while the extracellular space occupied 4.3% of the tissue volume in the controls and less than 0.5% in the stimulated brains. The partial volume of extracellular space obtained by extracellular markers and impedence measurements (2), however, is at least 15%, and the observed decrease in the extracellular space in the stimulated preparations is not nearly large enough to account for the simultaneous increase in the size of the post-synaptic elements. In morphometric measurements the volume fraction (V_V) of interstial space in tissue is equated with the area fraction (A_A) of the micrograph occupied by clear regions between cells. Because of the random oblique orientation of plasma membranes, the narrow regions of extracellular space are partially obscured. The diagram shows an edge view of a tissue section of thickness t in which two cells with parallel cell membranes, separated by a distance D, are oriented at an oblique angle θ to the plane of sectioning.



The apparent width of this extracellular space as observed in a micrograph would be W=Dsec θ-t tan θ. Since the actual cross-sectional area of the extracellular space is Dsec θ, the fractional underestimation of the extracellular volume is:

$$F = A_A/V_V = 1 - t \sin \theta/d \qquad (1)$$

If the cellular elements are assumed to be randomly oriented, then all values of θ from 0 to $\pi/2$ are equally likely and the expression for F may be integrated over all values of θ to give an equation for F $(\bar{d},t)$.(3) Although the distribution of D is not known exactly a mean value $\bar{D}$ may be assumed. Noting that the extracellular space in compact tissues is associated with the cell membranes as if it were a layer of thickness $\bar{D}/2$ on all cell surfaces, its volume fraction V_V, is then related to the surface density, S_V, of plasma membrane:

$$V_V = S_V D/2 \qquad (2)$$

Combined with equation (1) this expression yields $DF=2A_A/S_V$, where A_A and S_V are measurable quantities and $\bar{D}$ can then be determined from a plot of DF vs D. Using this result the actual volume fraction of extracellular space, calculated from (1) or (2) above, shows a value of approximately 20% (F=0.20) for control preparations and 5% (F=0.10) for KCl stimulated tissue. In addition, measurements of the membrane surface area end volume fraction of the tissue occupied by the pre-synaptic (axon terminals) and post-synaptic (dentritic spines) structures indicated that when stimulated with KCl, the extracellular space of the frog cortex is taken up by the dentritic spines which swell up and change shape-increasing their volume without a change in their membrane surface area.

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184 AN ULTRASTRUCTURAL INVESTIGATION OF THE EFFECTS OF MALNUTRITION ON THE CEREBRAL VASCULAR SYSTEM OF JUVENILE AND ADULT RATS. Elizabeth M. Burns. Dept. Med.-Surg. Nurs., College of Nurs. and Dept. of Physiol, Sch. of Basic Medical Sciences. U.I.M.C., Chicago, Ill. 60612

Previously healthy Sprague-Dawley female rats were given free access to isocaloric diets containing either 8% protein (malnourished) or 25% protein (well-nourished controls). The fat, vitamin and mineral contents of the two diets were identical. The dietary regime was initiated five weeks prior to mating and was continued to the dams throughout gestation and lactation, and to the offspring until studies were performed at 35 or 90 days postnatally. The severity of the nutritional deprivation is evidenced by the fact that the mean brain and body weights of 35 day-old malnourished animals were 80 and 20% respectively of well-nourished age mates.

After fixation of the brain by aortic perfusion, samples were obtained from the frontal cortex (area 8), striate cortex (area 17), parietal cortex (area 2) and from the candate-putamen. Full cortical thickness blocks of tissue, not greater than 1 mm in width, were post-fixed, dehydrated, and embedded in epon. Ultrathin sections, perpendicular to the pial surface, were made, stained on the grid with uranyl acetate and Reynold's lead, and photographed using the Phillips 300 electron microscope.

Ultramorphological characteristics of arterioles, capillaries and small venules from malnourished and well-nourished juvenile and adult rats are compared. Differences are noted with respect to thickness of vessel walls and numbers of mitochondria present in endothelial cells.

185 LIMB PREFERENCE AFTER CORTICAL LESIONS IN ADULT AND NEWBORN RATS. Anthony J. Castro. Department of Anatomy, Stritch School of Medicine, Loyola University, Maywood, Illinois 60153.

Limb preference was examined in rats after adult or neonatal cortical lesions. Similar to previous reports, unilateral frontal cortical lesions in adult rats resulted in an ipsilateral limb preference in all animals tested. This preference was not affected by preoperative and postoperative testing with a testing box that was designed to bias use of the limb contralateral to the lesion. However, large unilateral frontal cortical lesions, which generally included considerable damage to the caudate-putamen, in 1-2 day old rats did not produce the same uniform results when animals were similarly tested at 2-11 months of age. In this group of 16 animals, 8 of the animals tested demonstrated either an ambilaterality in limb preference (n=5) or a distinct preference for the contralateral limb (n=3). This group differed from those animals sustaining adult lesions in that limb preference in some of the animals was apparently influenced by the testing bias. Accordingly, these data suggest the possible involvement of a compensatory mechanism that was not present after adult lesions.

(Supported by NIH Grant NS13230.)

186 LOCALIZED AND DELOCALIZED POTENTIALS IN THE RAT BRAIN CORTEX. B. Chance, A. Mayevsky* and J. Smith*. Johnson Research Foundation, University of Pennsylvania Medical School, Philadelphia, PA. 19174 USA and Bar Ilan University, Ramat Gan, ISRAEL.

Fluorescent probes capable of exhibiting "electrochromic" indications can respond to localized or to delocalized potentials in membranes of a variety of well-characterized organelles (1,2). A particular example of this class is OX-V -- BIS-[3-Ø-5-Isoxazolone-(4)]-Pentamethine Oxonol. This probe responds to valinomycin-induced diffusion potentials in both linear and quadratic ways in phospholipid vesicles, submitochondrial particles, chromatophores, etc. (1,3). The probes are applied to the brain of the anesthetized rat, epidurally or intraventricularly. The fluorescence signals of OX-V are measured with 578 nm excitation and 620 nm emission by a light guide coupling to a cannula in the brain (4) within 20 minutes after application of the probe. The surface of the brain appears uniformly fluorescent and optical and electrical signals can be observed over the ensuing 24 hour period suggesting minimal toxicity and metabolic utilization. In anoxia and spreading depression (5), as the electrocorticogram disappears, decreases of the probe fluorescence are observed. The reappearance of 50-80% of the probe fluorescence occurs before the reactivation of the electrocorticogram significantly. Applications of this and similar probes to three-dimensional techniques where time- and space-resolved fluorescence maps of the probe in frozen trapped brain seem possible(6).

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187 MEMBRANE SURFACE CONTROL OF ION PERMEABILITIES. Steven D. Corbin* and Howard H. Wang. Biology Board of Studies, UCSC, Santa Cruz, CA 95064.

Miniature glass electrodes were used to record ion activities (sodium, potassium, calcium and hydrogen) in the cat cortex. Simultaneous measurements were taken of cortical impedance. Comparison of records obtained in the presence or absence of certain membrane-modifying drugs (ouabain, dilantin) as well as during the induction of asphyxia led to the following model: The anionic surface region of the neural membrane binds cations loosely, with a rapid turnover existing between free ions in the extracellular space and bound ions at the cell surface. A region is thus created at the cell surface through which cations must pass before they enter or leave the cell. the contents of the region are in a dynamic equilibrium which can be modified by a change in concentration of one or more of the ionic species. For an ion to enter the cell, it first binds in this region, then it diffuses into the cell. The experiments showed an interaction between calcium and sodium in the surface region; these ions bind diffusely and exist in dynamic equilibrium there. The equilibrium can be upset by hydrogen ion, but not by potassium. Changes in the concentration of either calcium or sodium will affect the binding of both and alter the equilibrium, thereby changing the rate of entry or egress as well as the ultimate intracellular or extracellular concentration.

188 RAPHE NUCLEI AND LOCUS COERULEUS PROJECTIONS TO FOREBRAIN IN RAT. James R. Couch and Phillip Black*. Dept. of Neurology, Kansas University Medical Center, Kansas City, Kansas, 66103.

In rats, horse-radish peroxidase (HRP) (Sigma, Type VI) was injected with a micro-syringe into occipito-parietal cortex (OPC) (3 animals), frontal cortex (FC) (1 animal) and caudato-putamen (CPU) (3 animals). A total of 1 mg. was injected in 10 µl over a 5-min period. After 24 hours animals were anesthetized with diabutal and perfused with a formaldehyde-glutaraldehyde solution followed by 5% sucrose solution. Sections were cut at 30 microns, mounted, and then incubated in 3,3 diaminobenzidine followed by exposure to H_2O_2. As controls, one animal had a sham injection of 10 µl of saline into OPC and another an intravenous injection of 12 mg. of HRP.

Neurons were identified by light microscopy as polygonal structures with granules of dark brown reaction product in the cytoplasm. After OPC injection, labeled neurons were identified in nucleus raphe pallidus (NRPa) and nucleus raphe magnus (NRM) of medulla, dorsal raphe nucleus (DRN), and locus coeruleus (LC) bilaterally as well as in lateral geniculate nucleus. After CPU injection labeled neurons were identified in NRPa and NRM and in LC bilaterally, as well as in substantia nigra. Only rare labeled neurons were seen in DRN after CPU injection. FC injection produced almost no brain stem labeling. The distribution of labeled neurons was generally sparse although occasional groups of neurons were seen. Almost no labeling was seen in median raphe nucleus or nucleus raphe pontis with any injection. No labeling of NRPa, NRM, DRN or LC was noted in control animals.

The data here support other work demonstrating widespread cortical projection of LC as well as DRN. The NRM and NRPa also appear to have widespread ascending projections including efferents to occipital cortex and basal ganglia.

189 SUBCORTICAL PROJECTIONS TO THE PREFRONTAL CORTEX IN THE RAT. Ivan Divac[1], Anna Kosmal[+2], Olle Lindvall[+3], and Anders Bjorklund[+3]. 1. Institute of Neurophysiology, University of Copenhagen, Denmark, 2. Nencki Institute of Experimental Biology, Warsaw, Poland, 3. Institute of Histology, University of Lund, Sweden.

Following injections of horseradish peroxidase into various neocortical areas of rat brains, labelled perikarya were found in a number of diencephalic and brain stem cell groups. The prefrontal cortex, defined by the projections from the mediodorsal nucleus of the thalamus, receives overlapping innervation from at least twelve other subcortical formations. These are the medioventral, parataenial, interanteromedial, posterolateral and intralaminar-midline thalamic nuclei, zona incerta, magnocellular nuclei of the basal forebrain, lateral and dorsomedial regions of the hypothalamus, claustrum, dorsal and central raphe nuclei, locus coeruleus and ventral tegmental area of Tsai. The prefrontal area is the only one innervated by the parataenial and interoanteromedial thalamic nuclei as well as by the presumably dopaminergic neurons of the ventral tegmental area.

190 POPULATION RESPONSE PROPERTIES OF NEURONS IN PREMOTOR CORTEX (AREA 6) OF THE CAT. Gernot S. Doetsch, Nelson Escobar*, & Hugh L. Norman*. Dept. Surg.(Neurosurg.) & Dept. Physiol., Med. Coll. Ga., Augusta, Ga. 30902.

Extracellular discharges were recorded from single neurons within the medial premotor cortex (area 6) in the precruciate gyrus of cats. Using electrical shocks to each of the four paws as "hunting stimuli," it was difficult to drive many neurons in this tissue; some were not responsive to any peripheral stimulation. Cells which could be activated formed a rather homogeneous subset (m neurons), responsive to electrical stimulation of each paw. About 7% of these cells were identified as pyramidal tract neurons. The m neurons were distributed widely throughout cortical depth, but were concentrated in layer V. After electrical stimulation of any paw, the m neurons responded with low mean probabilities (p=0.5-0.6), and with an average of 1.3-1.5 spikes per discharge; mean initial spike latencies were shorter after stimulation of the ipsilateral forepaw than the other paws. Using natural stimulation, most m neurons were found to have wide, bilateral receptive fields; cutaneous sensitivity varied throughout each field. About 40% of the cells responded only to hair movement, 30% to tapping or touching the skin, and 30% to both hair and tap stimulation; about 70% also responded to visual and/or auditory inputs. Following stimulation of the four paws, the population response patterns of the m neurons all showed peaks of activity within layer V; the patterns for each paw differed mainly in the timing of peak activity. It is concluded that the premotor cortex contains a subset of m neurons which receive convergent cutaneous and often polysensory inputs. These data support the view that gradients of neuronal subsets extend through different cytoarchitectonic fields of the sensorimotor cortex, with wide-field (m) neurons predominating anteriorly, and small-field (sa) neurons posteriorly. (Supported by GRS Grant 10-16-04-4010-23, Med. Coll. Ga.)

191 THE ORGANIZATION OF OPOSSUM SOMATIC SENSORY-MOTOR CORTEX. Ford F. Ebner, John P. Donoghue*, Robert Foster*, and Burgess N. Christensen. Neuroscience Sect., Brown Univ., Providence, R.I. 02912

This poster session will demonstrate the results of studies designed to analyze the connections of cells in forelimb area of somatic sensory-motor (SSM) cortex. The method of extracellularly injected horseradish peroxidase (HRP) was used for localization of afferent and efferent projection cells. For studies of cortical synaptic ultrastructure the method of iontophoretic injection of HRP or Procion brown (PB) into single, physiologically identified neurons has been employed.

Small cortical injections of HRP indicate that there are approximately twice as many cells filled in VB as in VL or intralaminar nuclei (CIN). HRP injections into subcortical sensory and motor structures have shown that cells located mainly in layer VI project to the thalamus, while fibers in the medullary pyramids arise from layer V neurons. Injections in the dorsal column nuclei also label cortical neurons in layer V; these cells are quite widely spaced in cortex.

The distribution of synapses is being studied on normal (control) neurons and on individually injected cortical neurons. The ultrastructure of pyramidal and non-pyramidal neurons in control cortex is comparable to previous descriptions in other mammals. There are few contacts on pyramidal cell bodies or their proximal dendrites. Following intracellular injection with either PB or HRP, neuronal processes could be traced to distal dendritic segments where synaptic types could be determined. The numerous spines on the distal segments receive mainly round asymmetrical contacts. These studies will combine the labelling of thalamic afferents with cortical cell injection in order to specify the position of thalamic terminals on the dendritic tree.
(supported by PHS grant no. NS 13031)

192 MESCALINE AND STRYCHNINE AS TOOLS TO STUDY THE LEVELS OF CORTICAL EXCITABILITY. Juan García Ramos. Dept. Physiol. Centro Investigación. National Polytechnic Institute, MEXICO

Topical application of either mescaline or strychnine to the cat's cortical surface gave rise to the local production of all-or-nothing spikes which can be elicited by afferent, antidromic, or direct electrical stimulation. These spikes showed a refractory state, are localized to the dendritic layer and usually gave rise to the firing of the pyramidal neurons involved. If evoked at regular intervals, these potentials were of constant amplitude which varied, however, with different types of afferent stimulation. They increased in those cases in which somatic afferent stimulation induced an awakening or alert cortical reaction, and decreased in amplitude when some interoceptive (baro- or chemoreceptors) afferents were stimulated. The increase or decrease in amplitude was present with corresponding changes in latency, in excitability, or in both.

The changes were better seen at very low levels of anesthesia, occurred in almost any region of the cortex, were established in a gradual way and took a relatively long time to go back to their control values. They are attributed to a humoral mechanism. The activating cortical reactions were mimicked by a catecholamine topically applied, and blocked by reserpine or the intracisternal administration of 6-hydroxydopamine. The depressant effects were mimicked by serotonine topically applied, and significantly reduced by a previous intracisternal injection of 5,6-dihydroxytriptamine.

193 AUTORADIOGRAPHIC DEMONSTRATION OF CORTICO-CORTICAL COLUMNS IN THE MOTOR, FRONTAL ASSOCIATION, AND LIMBIC CORTEX OF THE DEVELOPING RHESUS MONKEY. Patricia S. Goldman and Walle J. H. Nauta. NIMH, Bethesda, Md. 20014 and MIT, Cambridge, Mass. 02139.

The terminal distribution of cortico-cortical connections was examined by autoradiography following injections of tritiated amino acids into the dorsal bank of the principal sulcus, the medial orbital gyrus, or the precentral motor cortex of monkeys ranging in age from 4 days to 5½ months. Labeled axons originating in these various regions of the frontal lobe have topographically diverse ipsilateral and contralateral destinations but virtually all of these projections share a common mode of distribution: they terminate in distinct vertically oriented columns, 200-500 micra wide, that extend across all layers of cortex and alternate in regular sequence with columns of comparable width in which grain density does not exceed background. Spatial periodicity in the pattern of transported label in such regions as the precentral motor cortex, the prefrontal association cortex, and the retrosplenial limbic cortex indicates that columniation in the intracortical distribution of afferent fibers is not unique to sensory cortex but may be a common feature of neocortical organization in general.

A columnar intracortical distribution of cortico-cortical connections was noted at all ages investigated but the columns were especially circumscript and densely labeled in the youngest monkeys. The results of this study indicate that a columnar organization of some at least of the cortico-cortical afferents of the monkey's frontal and limbic cortex is achieved shortly after or even before birth.

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194 EFFECT OF PAPAVERINE AND EPINEPHRINE ON REGIONAL CEREBRAL BLOOD VOLUME (rCBV) DURING AN EVOKED FUNCTIONAL LOAD. Charles J. Hannan, Jr., Louis P. Gangarosa* and Jon Trueblood*. Pharmacology Dept., Medical College of Georgia, Augusta, Georgia 30902.

The relationship between a localized functional load to visual cortex produced by photic stimulation (PS) and vertebral artery infusions of epinephrine (Epi) and Papaverine (Pap) was explored in the rabbit with regard to changes in rCBV. A noninvasive method for rCBV determination in 0.1cc of tissue was based on X-ray activation of a nonradioactive intravascular tracer (see Phelps et al., 1973, J. Appl. Physiol. 35:741). This method allowed continuous evaluation of rCBV changes in 24 second intervals.

The question of whether vasodilators improve regional cerebral blood flow (considered estimated by rCBV) in ischemic or areas of compromised flow has been hotly debated. It must be concluded from our study that Pap does improve the perfusion of the brain to the extent that a local increase in rCBV in response to a functional load (PS) was not produced after Pap infusion. A summary of the temporal relationship between PS and rCBV change after drug infusions are below:

Seconds to significant increase in rCBV*

PS	PS & Epi	PS & Pap	PS & Epi
182 (5/5)	67(5/5)**	72 (1/5)	192 (1/5)

*mean from 5 experiments, parenthesis indicates animals responding
**$P < .02$, significant change from PS alone

Two relationships are evident: 1) a significant decrease in the latency for the rise in rCBV from PS after Epi infusion (37% faster); 2) only one of the five animals responded to PS with an increase in rCBV after Pap. This implies that the brain is perfused in excess of its needs after Pap. (Supported by USPHS-NIH Grant No. RO1 DE 03022)

195 THERAPY AFTER EXPERIMENTAL MIDDLE CEREBRAL ARTERY OCCLUSION IN MONKEYS: ULTRASTRUCTURAL FEATURES. Pamela K. Hill and Jack C. de la Torre. Mayo Clinic, Rochester, Mn. 55901 and University of Chicago, Chicago, Ill. 60637.

The transorbital surgical approach was used to induce experimental middle cerebral artery (MCA) occlusion in rhesus monkeys. This vascular insult injury mimics the occlusive cerebral infarction seen clinically. The monkeys received either no treatment, dexamethasone or DMSO. Ultrastructural findings of ischemic cortex suggest that monkeys receiving no treatment were the most severely affected by swelling of cytons and boutons as well as loss of synaptic vesicles and contacts. The cortex from dexamethasone-treated monkeys showed swelling of cytons and boutons with loss of synaptic vesicles but preservation of synapses. The extracellular spaces were dilated in both non-treated and dexamethasone-treated animals. The cortex from monkeys receiving DMSO displayed absence of cyton swelling, minimal bouton swelling with preservation of synaptic vesicles and contacts as well as absence of extracellular space expansion. On the basis of these findings, it was concluded that DMSO minimizes ultrastructural alterations following daily treatment for 4 days.

196 THE LAMINAR ORIGIN OF CALLOSAL AND ASSOCIATIONAL NEURONS TO THE PREFRONTAL GRANULAR CORTEX OF THE RHESUS MONKEY. Stanley Jacobson, Dept. Anat., Tufts Univ. Sch. Med., Boston, MA. 02111.

In order to identify the cells which form the converging multimodal input on the prefrontal granular cortex, Horseradish peroxidase (Sigma VI) was injected unilaterally into the cytoarchitectonic subdivisions on the convexity and medial surface of prefrontal cortex.

A heavy labeling of cells in all layers adjacent to the injection site was noted. Outside this narrow zone of presumed diffusion, short associational connections were seen in layers II-VI with the greatest number of cells in layer III in adjacent portions of prefrontal granular cortex. The cells forming the longer associational connections were found in cingulate areas 24 and 25, and in areas 21 and 22 in and around the superior temporal sulcus and in area 19 and 7. These cells were primarily in layer III with a few cells also seen in layers V and VI.

Callosal connections were also identified in homotypical and heterotypical cortical zones. The homotypical connections were seen in areas 8a,8b,9,10,11,12,45, and 46. The cells forming the homotypical connections were numerous and were found in layers II-VI being heaviest in III. The heterotypical connections were fewer in number and were seen in cingulate areas 24 and 25 and in areas 21 and 22 in superior temporal sulcus.

The cells which form the associational connections were small-medium pyramids. The cells which formed the callosal connections were small-medium pyramids, with some fusiform cells and even a few large granule cells in layer VI. The results of this study support the observations of the multimodal associational input onto the prefrontal granular cortex and also demonstrates that the callosal input also has multimodal converging input on prefrontal granular cortex.

197 PATTERNS OF DEPOLARIZATION WITHIN THE CORTEX BENEATH A SURFACE STIMULATING ELECTRODE. William B. Marks and Duen Yen*. Lab. of Neural Control, NIH, Bethesda, MD 20014 and Univ. of Vermont, Burlington, VT 05401.

The mechanism by which cells in the cortex are excited by surface stimulation is unknown. These computations show that the stimuli required to evoke phosphenes in humans (Brindley and Lewin, J. Physiol. 196, 479, 1968) or to excite the larger cortical neurons in cats (Pollen,BBE, in press); .2 ms pulses of 1 to 2 ma applied to a .5 mm disc electrode, should polarize the terminals of dendrites and axons that are within 1 mm of the electrode by 60 mv or more. Positive currents hyperpolarize the terminals and negative currents depolarize. The deeper parts of the same structures are oppositely polarized, but only 1/10 to 1/20 as much. These potential changes are given along space and time, and the effects of the position, orientation and shape of the structures stimulated, as well as the presence of myelin and changes in stimulus waveform, are estimated. Thus, although positive and negative currents are known to be about equally effective in producing phosphenes or firing large cells, the distributions of immediate depolarization they cause within the cortical structures, which may mediate these responses, are quite different.

198 EFFECTS OF VARIOUS INSPIRED GAS MIXTURES ON INTRACELLULAR OXYGEN TENSION IN INTACT CEREBRAL CORTEX. Michael H. Mitnick② and Frans F. Jobsis.①
Dept. Physiol. Pharm., Duke Univ., Durham, NC. 27710; ②Dept. Physiol. Div. Neurosurg., Univ. Penna. Sch. Med., Philadelphia, PA. 19174.

The effects of 5 minutes of inspiration of either 100% O_2 or 95% O_2 with 5% CO_2 on the intracellular oxygen tension (iPO_2) in intact cat cerebral cortex, under sodium pentobarbital (Nembutal) anesthesia, were examined. Non-invasive optical monitoring of iPO_2 was accomplished with the fluorochrome pyrenebutyric acid (PBA)①. The summary of the first series of respiratory experiments is presented in Table 1. As shown, the iPO_2 response to the gas mixtures was variable. These results suggest that accurate regional cerebral blood flow (rCBF) data, coupled with functional correlates of metabolic processes (such as EEG or evoked potential studies) are needed in addition to iPO_2 information for the accurate quantification of oxygen delivery and utilization in the intact, normally circulated brain.

Table 1

Exp't	iPO_2 Room Air mm/Hg	iPO_2 95:5% CO_2	iPO_2 100% O_2	% Change from Room Air 95:5% CO_2	% Change from Room Air 100% O_2
1	41.3	49.0	53.6	+19	+30
2	36.9	61.5	63.0	+67	+71
3	49.2	67.7	61.5	+38	+25
4	43.1	55.4	55.4	+29	+29
5	31.8	44.1	31.8	+39	N.C.

①Mitnick, M.H. and F.F. Jobsis: Pyrenebutyric acid as an optical oxygen probe in the intact cerebral cortex. J. Appl. Physiol., 1976, In Press.

199 CELLULAR ACTIVITIES RECORDED FROM SLICES OF HUMAN NEOCORTEX MAINTAINED IN VITRO. David A. Prince and Philip A. Schwartzkroin. Dept. Neurology Stanford Univ. Sch. Med., Stanford, CA. 94305

Extracellular and intracellular recordings were made from neurons in human neocortical slices maintained in vitro for 4-8 hours. Cortical biopsy tissue was obtained from patients undergoing partial temporal lobectomy and cut into 360 μ thick slices. Spontaneous cellular activity, and activity triggered by electrical stimulation near the pial surface or in white matter, were observed in all slices. These intracellular recordings represent the first such data to be obtained from neurons in the human CNS. Cells had average resting membrane potentials greater than 50 mV and generated overshooting spikes (65 mV or more) of short duration. Stimulation of the slice evoked both excitatory and inhibitory post-synaptic potentials, with EPSPs often triggering action potentials. Spontaneous discharge was blocked during IPSPs. Intracellular depolarizing current pulses evoked trains of action potentials followed by a membrane hyperpolarization; this hyperpolarization also interrupted spontaneous cell discharge. In 7 neurons a series of depolarizing and hyperpolarizing current pulses was presented; average input resistance was found to be 18 megohms and charging time constant 12.5 msec.

The number of human tissue samples studied remains too small to allow any correlation of cellular properties with electroencephalographic or neuropathological findings. However, these results do demonstrate the potential of the in vitro slice preparation for investigating neuronal characteristics in normal and abnormal human brain tissue.

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200 THREE-DIMENSIONAL MAPPING OF THE METABOLIC STATE OF THE RAT BRAIN. B. Quistorff*, B. Chance, W. Nadler*, S. Eleff*, and J. Sorge* (SPON: M. Reivich). Johnson Research Foundation, University of Pennsylvania Medical School, Philadelphia, PA. 19174 USA.

Three-dimensional mapping of the redox state of the rat brain under controlled physiological conditions has been persued for a couple of years (Chance et al., FEBS Mtg. Abstract, Paris, 1975; Quistorff et al., Proceedings of the APS Mtg., Anaheim, 1976). The ratio between the fluorescence of flavoprotein and pyridine nucleotide is used as an indicator of the regional redox state (Chance et al., Proceedings of the APS Mtg., Anaheim, 1976). The procedure for the read-out of these signals from morphologically well-defined single-tissue-volumes of about 10^{-3} mm^3 will be discussed: Part of the brain of a rat is sampled by the guillotine-freeze-clamping technique (Quistorff, Anal. Biochem. 68, 1976) which preserves the tissue morphology fairly well. The cross section of the freeze-clamped brain sample which have been in contact with one of the freeze-clamping blocks is scanned for the FP, PN and reflectance signals via 85 μ quartz fiber light guides. The scanning is performed by moving the light guide relative to the sample in steps of 100 μ, giving about 10,000 data points over the cross section of the brain. The optimal working distance for the light guide is 125 μ and at this distance the two-dimensional resolution is less than 100 μ. The third dimension is obtained by grinding away 0.1 mm from the sample surface by means of a special low-temperature surface miller, and repeating the two-dimensional scan on this surface.

The scanning and the reading procedure is computer-directed and the stored data can be displayed either as "redox ratio" picture or as an ordinary image, generated from the 366 nm reflectance measurements. 100 points/mm^2 makes it possible to recognize morphological details on the actual tissue surface. So morphology and metabolism can be linked by combining the information of the reflectance and the redox ratio images.

201 Diversity of Function and Variability in the Anterior Commissure of Man* Gail Risse*, Joseph LeDoux*, Sally Springer*, Donald Wilson* and Michael Gazzaniga. S.U.N.Y. Stony Brook.

The anterior commissure, which has been presumed to play a minor role in interhemispheric communication, was tested for the transfer of visual, auditory, olfactory and somatosensory information in patients with complete surgical sections of the corpus callosum. The S's were required to give a verbal (left hemispheric) description of modality specific information presented to the right hemisphere. The transfer capacity of each patient varied. While no S gave evidence of transferring somatosensory information interhemispheric communication in each of the other modalities was demonstrated in at least one case. These data strongly suggest that fibers of the anterior commissure are capable of transmitting diverse sensory messages. This conclusion is consistent with the anatomical distribution of the pathway, which implicates the older anterior limb in the exchange of information between paleocortical olfactory regions, and the more recent posterior limb in the transfer of visual and auditory impulses between temporal neocortical areas. The apparent ability of individual patients to transfer in one modality but not another can, in some cases, be explained by a pre-existing lesion involving the sensory system in question. Another, more intriguing possibility, however, is that functional and possibly even anatomical variation may exist in this commissural system. Although visual transfer via this route has been discussed for some time in the animal literature, and olfactory function has always been anatomically obvious, it is somewhat surprising that interhemispheric fibers from the superior temporal gyrus can sustain a functionally meaningful auditory exchange. In view of these findings, a revision of the significance of the role attributed to the anterior commissure in interhemispheric communication is clearly indicated. *Aided by USPHS Grant No. MH25643-02A1

202 THE SITE OF ACTION OF THE CORTICAL EFFECTS OF VAGAL AFFERENTS Hilda Rodríguez*, Ma. Cristina Eguía Lis* and Juan García Ramos. (Spon: J. Alanis R.). Centro de Investigación. National Polytechnic Institute, MEXICO.

Strychnine spikes elicited in the somato sensory area of the cat by single shocks to the superficial radial nerve or by direct electrical stimulation were used to ascertain the site of action of the cortical depression induced by vagal afferent stimulation. The records were taken on the oscilloscope with the sweep being driven in succession by both types of stimuli separated by 600 msec, the cycle repeated every 2 sec. The first stimuli in each cycle could be any one of the two. Amplitudes and latencies were measured before, during and after vagal stimulation of low threshold afferent fibers. Various other afferents were also used. The changes in amplitude and latency were similar and followed similar time courses for the two responses. A fact that was interpreted as indicating that the site of action of the afferent stimulation is at the cortical structures responsible for those potentials, considering that they are differently activated by the two modes of stimulation; one, through the sensory impulses reaching the cortex, the other by direct electrical stimulation. Those cortical structures would be the non-synaptic membranes of the apical dendrites. The systemic blood pressure was recorded, and cortical impedance and pO_2 measurements were made at the same cortical site, to control that the effects were not due to vascular changes of the pial vessels.

203 AFFERENTS TO AREA FL OF THE MEDIAL FRONTAL CORTEX FROM THE AMYGDALA AND HIPPOCAMPUS OF THE RHESUS MONKEY. D.L. Rosene*, M-M. Mesulam, and G. W. Van Hoesen. Harvard Neurological Unit, Beth Israel Hospital, Boston, MA.

Despite the widespread interest in the orbital cortex of the primate frontal lobe, very little attention has been paid to the adjacent cortex of the medial surface. Von Bonin and Bailey labelled a portion of this cortex area FL and described the features that distinguish it from the more dorsal portion of Brodmann's area 24 of the cingulate gyrus. FL is located ventral to the genu and rostrum of the corpus callosum and extends as far as the olfactory stalk and sulcus. It includes the gyrus rectus, the subcallosal portion of Brodmann's area 24 and most of Walker's area 25.

While there is very little data available on the functions of this portion of the frontal lobe, we can now report experimental evidence that this area receives afferents from the amygdala and the hippocampus. These experiments utilized rhesus monkeys as subjects and employed the Fink-Heimer, autoradiographic, and horseradish peroxidase neuroanatomical methods. Afferents from the amygdala originate in the basolateral nuclei and travel in the ventral amygdalofugal pathway to the frontal lobe. These afferents terminate throughout the extent of FL with the heaviest concentration in layers 1 and 2. The afferents from the hippocampus originate in the subiculum and travel in the fornix to the septal area where they course rostrally to enter FL. These afferents terminate primarily in the deeper layers throughout FL except at the apex of the gyrus rectus and immediately adjacent to the subcallosal hippocampal rudiment where heavy termination extends into the superficial layers.

Thus, in addition to the architectonic distinctions of Von Bonin and Bailey, area FL may be further characterized by its direct limbic afferents from both the hippocampus and the amygdala.

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204 ANTAGONISM OF BIOGENIC AMINE DEPRESSION OF RAT CEREBRAL CORTICAL NEURONES BY Na^+,K^+-ATPase INHIBITORS. B.S.R. Sastry* and J.W. Phillis. Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.

Recent reports indicate that brain Na^+,K^+-adenosine triphosphatase (Na^+,K^+-ATPase) is activated by noradrenaline, dopamine and 5-hydroxytryptamine. Furthermore, ouabain, barbiturates and ethanol, which reportedly inhibit Na^+,K^+-ATPase activity, have been shown to antagonize the depressant effect of noradrenaline on central neurones. The present iontophoretic studies were therefore undertaken in an attempt to determine whether the depression of rat cerebral cortical neurones by noradrenaline, histamine and 5-hydroxytryptamine involves activation of Na^+,K^+-ATPase. In male Sprague Dawley rats anaesthetized with methoxyflurane and nitrous oxide, the spontaneous discharge rate of deep cerebral cortical neurones was decreased by noradrenaline, histamine and 5-hydroxytryptamine. The depression was usually prolonged, lasting over 1-4 minutes following the termination of the biogenic amine application, and occasionally consisted of an initial short and a subsequent prolonged phase. The Na^+,K^+-ATPase inhibitors, ouabain, ethacrynic acid and harmaline, antagonized amine-induced depressions. This antagonism was rapid in onset, was often more pronounced on the late prolonged phase, and was absent 2-6 minutes following termination of the antagonist ejection. Reduction of cortical neuronal firing rates by γ-aminobutyric acid, adenosine, adenosine 5'-monophosphate and Ca^{++} was not altered by the above ATPase inhibitors. The results of this investigation indicate that depression of rat cerebral cortical neurones by the biogenic amines is likely to involve activation of Na^+,K^+-ATPase.

(Supported by the Medical Research Council of Canada.)

205 ULTRASTRUCTURE OF STRIATE CORTEX LAYER IV NEUROPIL IN SQUIRREL MONKEY. M. Tigges, J. Bos, J. Tigges and E. Breding*. Yerkes Regional Primate Res. Ctr. and Dept. of Anatomy, Emory Univ., Atlanta, Ga. 30322

An electron microscopic investigation of layer IV reveals an entangled mass of neuronal elements. As a first step in unraveling the synaptic organization, we identified different types of synapses and neurons and quantitatively counted them on photomontages covering large areas of neuropil. Of 2400 synapses, about 85% belonged to Gray's type 1, 15% to Gray's type 2. Both classes can be subdivided by size. Although the majority of both types is small (86% of type 1; 62% of type 2), the most conspicuous landmark of layer IV is the large type 1 terminal which often arises from a myelinated axon and establishes a number of en passage contacts before it terminates. After destruction of the lateral geniculate, terminals of this morphology degenerate. The large type 2 terminal also makes en passage contacts on its tortuous course through the neuropil. Neither presynaptic dendrites nor serial synapses were found. Compared to a subcortical visual structure, i.e. the superior colliculus, type 2 terminals constitute a much smaller percentage of the entire synapse population. This suggests a fundamental difference in the synaptic wiring and thus the processing of information in striate cortex versus subcortical visual structures. At least 2 types of neurons of distinct morphology and synaptic environment occur: small cells which receive only type 2 and large cells which receive both types of terminals onto their soma. (Supported by NIH Grants RR00165, EY00638, NS05244 and NSF Grant GB4270.)

206 CORTICO-PULVINAR NEURONS IN HETEROTYPICAL AND HOMOTYPICAL CORTEX OF THE MONKEY WITH A NOTE ON RECIPROCAL PULVINAR-CORTICAL CONNECTIONS. John Q. Trojanowski. Dept. Anat., Tufts Univ. Sch. Med., Boston, MA. 02111

Using autoradiography and the horseradish peroxidase method, the morphology and laminar distribution of cortico-pulvinar neurons in heterotypical and homotypical cortex as well as the reciprocity of connections between pulvinar and cortex was examined in five Rhesus monkeys which had received medial, lateral and inferior pulvinar nucleus injections of both tritiated amino acids and horseradish peroxidase.

Cortico-pulvinar neurons were identified in one heterotypical cortical area (area 17) and in many homotypical areas in frontal (areas 45,46,11, 12), parietal (areas 5,7), occipital (18,19) and temporal (areas 20,21,22) lobes. The cortico-pulvinar neurons were pyramidal in shape and ranged in size from small to large. In heterotypical cortex they were found in layers V and VI whereas in area 17 they were found only in layer Vb. In homotypical cortex some of the labeled neurons located in the deep parts of layer VI and subjacent white matter were noted to be horizontally oriented. Reciprocal connections between pulvinar and cortex were a feature of homotypical but not heterotypical cortex.

The organization of the reciprocal connections between pulvinar and homotypical cortex differs from that of the specific thalamic relay nuclei and their related heterotypical cortical areas. Additional information regarding these differences should help to clarify how specific structural organizations form the substrate for different physiological functions.

207 CHARACTERIZATION OF INTRACELLULAR RECORDINGS FROM NEOCORTEX OF AWAKE CATS. II. RESPONSE TO CURRENT INJECTION. C. D. Woody and E. Gruen*. Depts. of Anatomy and Psychiatry, UCLA Medical Center, Los Angeles, Ca. 90024.

Initial characterizations were performed three years ago in 221 units with spike potentials (AP) of $\geq$ 20 mV amplitude and associated baseline potential shifts (MP) on penetration of comparable or greater magnitude (Woody and Black-Cleworth J. Neurophysiol. 36: 1104-1116, 1973). Present studies were performed over a period of 4 months in 179 additional units using 20-50 MΩ glass electrodes filled with 1.4M K^+ citrate plus 5 μM cyclic GMP or 5' GMP (guanosine monophosphate). Capacitance compensation was carefully controlled. Previous findings were reproduced using these electrodes. 72% of encountered units had AP < 40 mV; none had AP > 75 mV. Of 74 cells with MP $\geq$ 48 mV, only 35 had AP $\geq$ 48 mV. 51 had MP between 48 and 51 mV. Injection of either rectangular pulse (10 msec at 100 Hz) or steady depolarizing currents in both AP "overshoot" and AP "undershoot" neurons resulted in decreased size and broadening of AP with increased rate of discharge. An inverse, linear relationship between AP amplitude and the amount of intracellularly injected current was found for depolarizing currents up to 3 nA. This relationship can be used to assess cell penetration and changes in cell input resistance. Injection of hyperpolarizing currents caused prompt cessation of discharge with rapid recovery afterwards. "Undershoot" neurons show simple or ceiling responses to ramp depolarizing currents as do cells with larger AP (R. Morita, unpublished observations), require slightly greater currents (mean difference, 0.2 nA) for initiation of spike discharge during pulse delivery than do "overshoot" units, and have PST histograms like those seen with extracellularly recorded units. The above results, plus observations after dye injection by Houchin (J. Physiol. 232: 67-69, 1973), support the view that undershoot recordings reflect penetration of dendritic regions remote from sites of spike initiation. (Supp. by USPHS HD-05958, HD-04612)

Chemical Senses: Smell and Taste

208 DIFFERENTIATION OF OLFACTORY RECEPTORS IN COMBINATION WITH OLFACTORY BULB IN ORGAN CULTURE. Albert I. Farbman* (SPON: M. F. Orr). Dept. Anat., Northwestern Univ. Med. School, Chicago, IL. 60611

In previous studies in this laboratory it was shown that explants of nasal placode or nasal pit taken from 11-13 day rat fetuses and grown in culture for one week contained well differentiated olfactory receptor cells. Moreover, when these cultured explants were stimulated with odorants, such as amyl acetate, methyl benzoate and others, electro-olfactograms could be recorded from them (Farbman and Gesteland, 1975). In the present study, nasal pit explants from 12 and 13 day rat fetuses were cultured with fragments of brain from the region closest to the roof of the nasal pit (presumptive olfactory bulb = POB). At this stage of development the POB resembles a pseudostratified epithelium and is separated from the presumptive olfactory epithelium (POE) by a narrow band of mesenchyme. There were three groups of cultures: 1) fragments of POB alone, 2) fragments including the nasal pit attached by mesenchyme to POB, 3) fragments of POB dissected free from the nasal pit and explanted adjacent to it. All explants were placed on collagen coated Millipore filters on a stainless steel grid platform in Falcon culture dishes. The medium was Waymouth's MB 752/1 supplemented with 15% newborn calf serum and cultures were grown in an atmosphere of 5% CO_2 in air at 34°C. After 6-8 days the specimens were processed for routine histological examination. In all of the POB explants, there was lamination of cells into two major groups: a band several layers deep of small, closely packed cells with circular darkly stained nuclei; these cells remained close to the free surface of the original cerebral vesicle. Distant from the surface were larger cells with paler staining nuclei. These cells were less closely packed and variable sized bundles of nerve fibers passed among them. The two cell types resemble the granular and mitral cells of olfactory bulb. In both groups of explants in which POE was combined with POB, nerve bundles could be traced from the base of the olfactory epithelium into the brain tissue. In some specimens small glomerulus-like structures were seen in the brain part of the explant. These preliminary results indicate that this method can provide a model system for studying differentiation of the olfactory bulb and early synaptogenesis of peripheral receptor cells with the bulb.

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209 THE CHEMICAL SENSES INVOLVED IN GARTER SNAKE PREY TRAILING. John L. Kubie* and Mimi Halpern (SPON: S. Balagura) Prog. in Biol. Psych., Downstate Med. Ctr., S.U.N.Y., Brooklyn, N.Y. 11203.

Garter snakes will follow prey trails of aqueous earthworm extract in a maze and, while following these trails, will exhibit the rapid tongue flicking and pendular head movements which are typical behaviors of snake trailing (Kubie and Halpern, J. Comp. Physiol. Psych., 89: 667, 1975). Fifteen snakes of the genus Thamnophis were pretrained to follow earthworm extract in a four-choice maze for worm bit rewards. They were then tested for their abilities to accurately follow seven concentrations of wet earthworm extract (wet trails), earthworm extract dried on tape (dry trails), earthworm extract dried on tape and rewet with distilled water (dry-rewet trails), and earthworm extract laid below a perforated floor (removed trails). All snakes exhibited high tongue flick rates to the trails they followed most proficiently. Snakes trailed the high concentrations of wet extract with greater than 90% accuracy, and their performance fell off with weaker dilutions. All snakes were able to follow the dry and dry-rewet trails, averaging 67.5 and 82.5% correct respectively. Trailing accuracy on dry-rewet trails is significantly better than trailing accuracy on dry trails ($t=5.4$, $df=8$, $p<.001$). Only one snake gave any evidence of following the removed extract trails. Apparently, a snake has to make direct lingual contact with the extract to utilize the trail for locating prey.

After baseline testing eight of the snakes were given one of three surgical procedures: bilateral vomeronasal nerve cuts (n=4), bilateral olfactory nerve cuts (n=2) or sham nerve cuts (n=2). Snakes with vomeronasal nerve cuts trailed at chance levels postoperatively. Their tongue flick rates were low and did not vary with the strength of extract trail. Two of these snakes began to spit out earthworm bits during the first few postoperative days, and, shortly thereafter, they stopped attacking the earthworm bits altogether. Snakes with olfactory nerve cuts and snakes with sham nerve cuts continued to follow all trails at preoperative levels. Their tongue flick rates remained high when following the stronger extract trails.

Snakes in the sham and olfactory nerve cut groups then had the vomeronasal ducts in the roof of the mouth sutured shut. After the duct suture these snakes were unable to follow any of the extract trails at better than chance levels, but, in contrast to the snakes with vomeronasal nerve cuts, most of these snakes continued to attack and ingest earthworm bits.

These results suggest that garter snakes are heavily dependent on their vomeronasal systems for following chemical prey trails and that the tongue must make direct contact with the trail to deliver the appropriate odorants to the vomeronasal organ. In addition, tongue flick rates during prey trailing appear to be directly related to the amount of vomeronasal stimulation the snakes receive.

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210 A THEORETICAL MODEL OF SENSORY TRANSDUCTION IN CHEMO-RECEPTORS.
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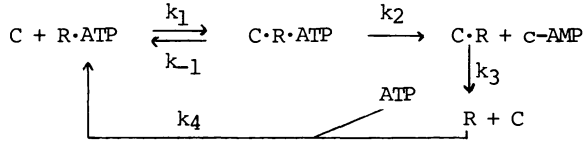
In 1954, Beidler proposed that sensory transduction in taste receptors depends on an initial reaction between the chemical stimulus and a specific taste receptor, according to the reaction $C + R \rightleftarrows CR$, and assumed that the response to stimulation (R) is proportional to the number of bound receptors (CR). On the basis of this model, he derived the fundamental taste equation, $C/R = C/Rm + 1/KRm$ which predicts a linear relationship for a C/R versus C plot. However, Beidler's model does not predict the initial phasic component of the temporal response to continuous stimulation, as recorded intracellularly in the chemo-sensory hairs of flies by Morita and Yamashita (Science, 130:992, 1959).

In this report a modification of Beidler's fundamental taste model is described which predicts the phasic response, and generates linear C/R versus C plots. The model is also consistent with the requirement for an internal transmitter, as suggested by Beidler (1970), which is assumed to be cyclic-AMP on the basis of experiments described by Smith and Friend (J. Insect. Physiol. 18:2337, 1972). The proposed model is diagrammed as follows,



where C is the chemical stimulus, R is a specific taste receptor with a binding site for ATP on the inner surface of the taste cell membrane, and a binding site for C on the external surface. Cyclic AMP (c-AMP) is assumed to function as an internal transmitter, initiating excitation of the receptor cell.

In the absence of stimulus (C) the receptor (R) binds ATP to form an active receptor (R-ATP). On contact with stimulus, c-AMP is produced at a rate dependent on the concentration of C, thereby generating the initial phasic response. As active receptors are regenerated by the recovery steps, 3 and 4, the sustained or tonic response is produced. Adaptation of the response following application of concentrated stimulus, or prolonged stimulation, is assumed to result from depletion of ATP for regeneration of active receptors.

Thus, the model predicts the phasic and tonic components of the temporal response to continuous stimulation, as well as sensory adaptation. Analog computer simulation of the model confirms these results, and generates linear C/R versus C plots in agreement with the fundamental taste equation. The steady-state equation based on this model,

$$R = \frac{Rm[C]}{(k_{-1} + k_2)/(k_1) + (1 + k_2/k_3 + k_2/k_4[ATP])[C]}$$

shows the dependence of the response R on stimulus concentration [C], where Rm = maximum response. In linear form, this equation becomes $[C]/R = (1 + k_2/k_3 + k_2/k_4[ATP])\ [C]/Rm + (k_{-1} + k_2)/(k_1)\ 1/Rm$ which is analogous to the fundamental taste equation, and shows a linear relationship for C/R versus C plots provided that the ATP level remains constant. At the peak of the initial phasic response, this equation reduces to $[C]/R = [C]/Rm + 1/KRm$, where $K = (k_1)/(k_{-1} + k_2)$ which is identical to the fundamental taste equation except for the term k_2 in the kinetic constant, K.

These equations predict that the slope of a C/R versus C plot should be lower for the phasic response than for the tonic response. Experimental studies confirm this prediction.

The proposed model represents a logical extension of Beidler's fundamental theory of taste, and agrees with current views pertaining to the mechanism of sensory transduction in chemo-receptors.

211 A MEASURE OF EXTRACELLULAR UNIT RESPONSES TO OLFACTORY STIMULATION APPLIED TO THE PROBLEM OF OLFACTORY HABITUATION. John W. Scott* (SPON: W. K. O'Steen). Emory University, Atlanta, Georgia 30322

Observations by several authors have pointed out the inadequacy of mean frequency measure (ie. number of spikes during a period of stimulation divided by the length of the period) in quantifying responses of single neurons of the olfactory system to odor stimulation. Several types of temporal patterns of spike activity during odor stimulation have been described, but no analysis has been developed for comparison of magnitude of response when different patterns of response are seen. This paper proposes an analysis for repeated presentations of an olfactory stimulus where the timing of the odor inhalations (or sniffs) is fixed. In this work the sniff period was fixed at 2 sec. and divided into ten 200 msec. bins. A measure of the pattern of activity was given by the sum of the products of the number of counts in each successive bin of a pair of sniff periods multiplied by the number of bins and divided by the total number of spikes in each of the two sniff periods:

$$G(J) = \frac{K \Sigma f(n_J) f(n_{J+1})}{\Sigma f(n_J) \Sigma f(n_{J+1})} .$$

This measure interrelates the Jth and J+1th sniff periods. K is the number of bins. The quantities $f(n_J)$ and $f(n_{J+1})$ are the number of spikes in the nth bin of the Jth and J+1th sniff periods. All sums are from n=1 to n=K. G(J) is greater than 1 if a non-uniform temporal pattern exists that is similar in the two sniff periods, is equal to 1 if a uniform temporal pattern exists in one of the cycles and is less than 1 if the patterns are non-uniform in dissimilar manners (ie. if temporal patterns uncorrelated to the sniff period exist). Thus G(J) greater than 1 indicates the presence of a stable pattern in the spike activity. G(J) is not sensitive to mean frequency (F(J) = $f(n_J)$/ length of sniff period).

The activity during odor stimulation was characterized by combining the pattern and mean frequency measures. The value for each measure for each sniff period was compared with average control or unstimulated values by the following expression:

$$V_J = \sqrt{\left\{\frac{F(J) - F(J)_c}{F(J)_c}\right\}^2 + \left\{\frac{G(J) - G(J)_c}{G(J)_c}\right\}^2} ,$$

where $F(J)_c$ and $G(J)_c$ are the respective control values.

Examples are given of the sensitivity of this procedure for quantification of various patterns of response at varying stimulus intensities. The procedure is applied to data from 29 olfactory bulb units and 17 lateral hypothalamic units observed under urethane anesthesia. It is shown that by the measure V_J the hypothalamic units habituate significantly faster. The habituation of hypothalamic units responses is influenced by factors which desynchronize the EEG.

212 FROG NASAL CAPSULE: VOLUME AND SURFACE AREA MEASUREMENTS IN RANA CATESBIANA AND R. PIPIENS. David P. Bashor, Dept. of Biol., UNC at Charlotte, N.C. 28223.

Since olfactory stimulation involves mass transport and sorption phenomena, it is useful to know the total sorptive surface area and gas volume contained within the nasal cavity. The cavity is covered by a continuous sheet of mucus, so its surface may be treated as if smooth. Nasal cavities of the frogs Rana catesbiana (550-750 g body weight) and R. pipiens (60-100 g body weight) were measured from camera lucida drawings of stained histological sections. Cavity perimeter of sections was determined either by weighing the traced cavity cut from paper or by means of a planimeter. To correct for an estimated 20% shrinkage in linear dimension, measured lengths were multiplied by 1.25, areas by 1.56, and volumes by 1.95.

	R. pipiens	R. catesbiana
Mean Capsule volume	.02 cm^3 (n = 2)	.25 cm^3 (n = 2)
Mean Capsule surface	.33 cm^2 (n = 2)	3.63 cm^2 (n = 2)
Surface/volume ratio	16.5	14.5

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213 COMPARISON OF CAT AND HUMAN TASTE RESPONSES TO AMINO ACID SOLUTIONS. James C. Boudreau. Sensory Sciences Center, University of Texas Graduate School of Biomedical Sciences, Houston, Texas 77030.

A quantitative comparison was made between human and cat taste responses to L-amino acid solutions. Neurophysiological measures from the cat (single unit recordings from the geniculate ganglion) were compared with psychophysical responses for humans (Nimomiya et al., 1966). Amino acids elicit complex sensations from humans although the sensations of sweet, bitter, and sour predominate. Of the three functional chemoresponseive neural groups identified in the cat geniculate ganglion, only groups I and II are discharged by amino acid solutions. Group I units are discharged by those amino acids that elicit a human sour sensation. Group II units are inhibited by the amino acids that elicit a strong bitter sensation and are excited by those tasting sweet, or sweet and bitter; though the best group II stimuli are not the sweetest compounds. The neural and psychophysical responses to the amino acid solutions can be partly interpreted in terms of the chemical properties of the side chains. Group I stimuli and sour solutions contain amino acids with acidic side chains, where an acid is defined as a Brønsted acid (thus L-histidine is a stimulus due to the proton donating characteristics of the imidazole group). Group II unit inhibition and bitterness are directly related to the hydrophobicity of the amino acid side chain, where hydrophobicity is calculated according to Tanford's hydrophobicity scale. This research was supported in part by NSF Research Grants.

214 MORPHOLOGICAL AND QUANTITATIVE STUDY OF DEVELOPING EPIGLOTTAL TASTE BUDS IN SHEEP. Robert M. Bradley and MaryLou Cheal. Dept. Oral Biol., Sch. Dent., Univ. Michigan, Ann Arbor, MI 48109.

To better understand the maturation of laryngeal reflexes, a quantitative study was made of developing epiglottal taste buds in sheep. The epiglottis was dissected from 17 fetuses aged 67 days to term (147 days), 8 lambs and 4 adult sheep. The tissue was fixed in neutral buffered formalin, prepared for light microscopy and stained with H & E. Taste buds are first seen on the epiglottis at about 80 days of gestation. They pass through a number of morphological stages before assuming the adult form. Initially taste buds do not have a taste pore, but by 125 days they resemble adult buds in that they possess: a pore; cells oriented perpendicularly to the basement membrane; and a characteristic shape. Later in gestation, at ~ 125 days, the taste buds enlarge and many buds have multiple taste pores. These large, multi-pored buds are possibly undergoing division, thereby increasing taste bud numbers. In lambs of 15 days and older the multi-pored structures are not observed; rather the taste buds are similar to those seen in adults.

There is a progressive increase in taste bud number with gestation. Up to ~ 125 days of gestation the increase results from de novo differentiation of taste buds. Further increase in taste bud numbers may result from a division of existing multi-pored taste buds. The latter suggestion is supported by the fact that no new, immature taste buds appear after ~ 125 days of gestation. After birth the number of taste buds is so variable among specimens that it is not yet possible to resolve whether taste bud numbers are increasing, decreasing or remaining the same. It is concluded (1) that there are two processes involved in epiglottal taste bud formation; and (2) that the individual differences in taste bud number after birth might contribute to variability in upper airway reflex patterns.
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215 SPECIALIST AND GENERALIST TASTE FIBERS IN THE CATFISH. John Caprio* and Don Tucker. Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803 and the Dept. of Biological Science, Florida State University, Tallahassee, Florida 32306.

Single unit analysis of in vivo barbel nerve preparations from the channel catfish (Ictalurus punctatus) revealed that gustatory fibers can be classified into at least 2 categories, alanine-best and arginine-best fibers. Of the 20 single fibers tested, 6 were alanine-best and 14 were arginine-best units. Gustatory single fiber responses to amino acid concentration series were more variable than observed for the whole nerve bundle (Caprio, Olfaction & Taste V) and thresholds ranged between 10^{-4}M and 10^{-11}M. The response rate of the majority of both fiber types increased exponentially with logarithmic increase of concentration, similar to the whole nerve preparation, and some fibers responded over 7 log units of stimulus concentration (mean, 4.7±1.4). Amino acid specificities of individual units were characteristic of the fiber type. The alanine-best taste fiber, a generalist fiber, responded to all the amino acids tested at 10^{-4}M or 10^{-3}M. The order of effectiveness of amino acid stimuli for an alanine-best unit was similar to that for the whole nerve response, except the unit response to arginine was less than predicted from the whole nerve activity. However, the arginine-best taste fiber, a specialist fiber, was highly specific and responded primarily to L-arginine, L-arginine methyl ester, D-arginine, and slightly to L-alanine. Unit responses to amino acid esters suggest the ester may bind to the same receptor site as the amino acid and that an ionically charged carboxyl group is unnecessary for receptor response. The present category of alanine-best and arginine-best taste fibers adequately describes the population data that have been obtained.
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216 NEURAL RESPONSES OF NORMAL AND REGENERATED TASTE FIBERS IN GERBILS. MaryLou Cheal, William Dickey*, Lee B. Jones*, and Bruce Oakley. Div. Biological Sciences, Univ. Mich., Ann Arbor, MI. 48109.

Interruption of the chorda tympani nerve caused fungiform taste buds to degenerate ipsilaterally. Axons readily regenerated to the tongue and reformed taste buds. At 11-16 days following surgery, when responses could be recorded from the chorda tympani nerve, single and few unit taste responses had the following properties: 1) Most fibers were spontaneously active. 2) Chemical stimulation produced an irregular discharge with an initial transient followed by adaptation. 3) The firing rate increased with increasing stimulus concentration. 4) Fibers had both narrow and broad response profiles; i.e., fibers might respond to a few or to several of the 13 chemicals tested. The multiplicity of responses from newly regenerated axons reflects the normal diversity of single units. We found similar response properties (1-4) in a population of 26 normal fibers. Receptive field properties of the regenerated fibers were also determined: 1) Each fiber's receptive field was limited to a small number of fungiform papillae. 2) Two different papillae innervated by branches of the same axon responded to the same chemicals. 3) When receptive fields were localized there was always at least one taste bud present in that field. 4) Responses were not obtained in the absence of taste buds nor from the areas between fungiform papillae. We conclude that responses of newly regenerated taste fibers were mediated by regenerated taste buds and were qualitatively similar to those of normal mammalian taste fibers. (Supported in part by USPHS Grant NS-07072.)

217 SPECIFICITY IN OLFACTORY DEVELOPMENT: BEHAVIORAL EVIDENCE Catherine Cornwell-Jones*, Sonya K. Sobrian (SPON: Paul D. Mabry), Dept. Psych., Princeton Univ., Princeton, N.J. 08540.

The odor preferences of Wistar and Sprague-Dawley rat pups, 3-16 days old, were measured in a 2-choice situation preventing tactile or gustatory cues and requiring a locomotor response. The results imply specificity of olfactory development in two respects. First, within each strain, responses to two different odors--lemon and home nest shavings--appear to develop independently. The developmental dissimilarities suggest that different mechanisms mediate behavior guided by the two scents. Second, differences between strains were obtained in the ontogeny of response to lemon but not nest odor. Thus, olfactory mechanisms underlying responses of these two strains to an arbitrary, ethologically irrelevant odor seem to have drifted apart in their development. In contrast, the substrates mediating pheremonally-guided behavior appear to retain developmental similarities in that nest odor attracts both strains by day 4. Other studies investigating the ontogeny of rat social odor preferences have employed various procedures and strains. This diversity has prevented a general developmental pattern of pheremonally-guided behavior from emerging. The maturational parallels observed between Wistar and Sprague-Dawley pups in the present study suggest that procedural differences have previously obscured this similarity across strains.

218 EVIDENCE THAT SECOND-ORDER OLFACTORY NEURONS CAN ENCODE "ACROSS-FIBER" PATTERNS IN THE OLFACTORY NERVE. William A. Corrigall and Marvin H. Sherebrin*. Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. and Dept. Biophysics, Univ. Western Ontario, London, Canada.

Single unit extracellular spikes have been recorded from second-order olfactory neurons of the frog. Electrical stimulation of the olfactory nerve could result in invasion of the sampled neuron by a spike at constant latency (ca. 20 msec), consistent with the slow conduction velocity in these fibers. Even when this orthodromic spike could not be elicited, spontaneous activity in the second-order neurons (Corrigall and Sherebrin Br. Res. 103:555,'76) could be modified by stimulation; response patterns involved depression of the spontaneous activity and high frequency spike discharges similar to patterns observed following odorant stimulation of the olfactory mucosa (e.g., Doving, Acta Physiol. Scand. 60:150,'64).

The components of these responses could, on occasion, be incorporated differentially by increasing the stimulus magnitude. At low stimulus currents the response was a single spike at latency typical for orthodromic invasion. As the stimulus current was increased further, the latency of this single early spike remained constant, but additionally a burst of spikes was produced whose number increased and whose latency decreased with increasing stimulus magnitude. If one considers the primary result of increasing the stimulus magnitude to be activation of greater numbers of olfactory nerve fibers, then these second-order olfactory neurons are encoding a spatial array of nerve responses into a temporal sequence of spikes. At least some second-order olfactory neurons may, therefore, be capable of encoding the "across-fiber" receptor response proposed by O'Connell and Mozell (J. Neurophysiol. 32: 51,'69).

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219 RECEPTIVE FIELDS OF SECOND ORDER CELLS IN THE OLFACTORY BULB OF THE HAMSTER. Richard M. Costanzo. The Rockefeller Univ., New York, NY 10021.

Electrical stimulation of olfactory nerve fibers emerging from different positions along the olfactory mucosa was used to map both the location and size of the receptive fields of cells in the olfactory bulb of the hamster. Single units were recorded extracellularly with metal filled glass micropipettes. Units recorded from lateral regions of the bulb responded to stimulation of lateral positions in the olfactory mucosa but not to stimulation of medial positions. On the other hand units recorded from medial regions of the bulb responded to stimulation of medial, but not lateral positions in the olfactory mucosa. The size of a given unit's receptive field was determined by the response to stimulation of a series of mucosal positions (approximately 250μ apart) arranged across the receptor sheet. Most of the units tested were found to have very localized receptive fields limited to only two or three adjacent positions. A previous study in the frog (Costanzo and Mozell, 1974) demonstrated a topographical projection of the olfactory mucosa onto cells in the olfactory bulb. This finding has now been confirmed and extended in the hamster where the structure of the nasal cavity is considerably more complex. The present results demonstrate that the location of a unit's receptive field in the olfactory mucosa is highly correlated with the location of the unit in the olfactory bulb. Furthermore, the receptive fields of mammalian bulbar units appear to be more localized than those in the frog. It is conceivable that these small, localized receptive fields for second order olfactory neurons play an important role in olfactory discrimination. (Supported by NIH 1-F22-NS00297-01 and NS 08902.)

220 INVESTIGATION INTO THE MECHANISM OF THE WATER RESPONSE. Janice A. Coté* and Michael B. Wang. Dept. Physiol., Sch. Med., Temple Univ., Phila., Pa. 19140.

Distilled water, when applied to the surface of the frog's tongue, causes an increase in the firing rate of single glossopharyngeal nerve fibers. It is unlikely that water produces this effect by interacting with a water binding site or by acting as an osmotic stimulus. It also seems unlikely that, as suggested by Nomura, H. and Sakada, S. (Jap. J. Physiol. 15: 433, 1965.), the water response is a response to calcium in the water, since the calcium concentration in the water which was flowed over the tongue was less than 10^{-12} molar. Finally, the response is not due to calcium entering the cell, since verapamil and manganese chloride, which block calcium influx across cell membranes, fail to prevent the response to water. Lanthanum chloride, which replaces calcium at external binding sites, completely and reversibly inhibits the water response. Sodium chloride reduces the water response in a dose dependent way, suggesting that water elicits a response by washing sodium ions from binding sites on the taste cell membrane. The removal of these sodium ions may activate the receptor, and provided calcium is bound to its binding site, a neural response occurs. (Supported in part by GIA Grant # 700-050-67).

221 UNIT DISCHARGES IN THE MEDIODORSAL NUCLEUS OF THE OPOSSUM EVOKED BY ELECTRICAL STIMULATION OF THE LATERAL OLFACTORY TRACT. Gregory T. Golden*, Jan C. Jackson* and Robert M. Benjamin. Dept. of Neurophysiology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

Discharges of single cells in the thalamic mediodorsal nucleus (MD) of the opossum were recorded during electrical stimulation of the lateral olfactory tract. Responsive sites were histologically localized throughout the entire mediolateral extent of MD which is uniformly magnocellular in appearance. In both rabbit and squirrel monkey responses were confined to the medial half of MD which in the monkey, at least, is distinctly magnocellular. The lateral parvocellular division was unresponsive. Thus the lateral nonolfactory nuclear subdivision, common to both rabbit and squirrel monkey, was not found in the opossum.

Firing patterns of cells were similar to those observed in rabbit and squirrel monkey. They commonly consisted of an early spike or burst of spikes, followed by a period of inactivity and, in many cells, by a later period of response or of resumed spontaneous activity. Latency distributions for the early discharges were comparable across species and indicate that relatively direct olfactory input is characteristic of MD in a diverse sample of mammals.

222 THE PALATAL AND LINGUAL DISTRIBUTION OF RAT GENICULATE GANGLION NEURONS. Maximo M. Gomez. Dept. Anat., Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103.

Classically, the geniculate ganglion (GG) of the facial nerve has been described as being comprised of cells that send their peripheral processes to the anterior tongue via the chorda tympani (CT) nerve, to the palate via the greater superficial petrosal nerve (GSP), and to the external ear via the posterior auricular nerve. Neuron counts were made in 10 GG which contained from 1597 to 1866 neurons with an average of 1721 cells. In addition, axons were counted with the electron microscope in one GSP; a total of 2376 axon profiles were seen. Of these 1703 or 72% were unmyelinated and only 673 or 28% were myelinated; all of the profiles were less than 4.0 μ in diameter. The sensory fibers in the GSP supply taste buds (TB) on the rat palate which average 125 TB per ipsilateral palate. By comparison, Beidler's CT study showed an average of 989 axon profiles in 4 CT, 60% of which were myelinated and 40% unmyelinated; the CT distributes to an average of 93 TB on the ipsilateral anterior tongue. Thus, there is a comparable number of myelinated fibers per TB in the CT (6.4) and GSP (5.5). The significance of the large differences between the numbers of unmyelinated fibers in the CT and GSP is unclear. The total number of axons in the GSP and CT together is greater than the number of GG somata. This peripheral fiber excess may be due to the presence of axons whose cell bodies reside outside of the GG (e.g. autonomic efferents) or to branching of the peripheral processes of GG cells. Studies are in progress to identify the GG neurons which give rise to CT and GSP fibers in order to further quantify facial nerve gustatory innervation. (Supported in part by NIH grant NS 10389.)

223 CHARACTERISTICS OF THE HYPOTHALAMIC PROJECTIONS TO THE OLFACTORY SYSTEM. R. Guevara-Aguilar, A. Nuño* and H.U. Aguilar. Depto. de Fisiología, Fac. de Med., Univ. Nacl. de México, México 20, D. F.

In a previous experiments performed in cats it was found that the electrical stimulation of the ventro-postero-lateral area of the hypothalamus evoked in both olfactory bulb potentials with three negative components and latencies of 2-4; 20-40 and 40-80 msec. However it was not possible to identify which pathways followed the afferent fibers.

Thus a new series of experiments in cats were performed. After recording the above mentioned evoked potentials for some minutes surgical or electrolitic lesions were performed. The section of the corpus callosum, the anterior commissura and the supramamillar decussation decreased the magnitude of the first component and almost abolished the 2nd and 3rd component. The first component clearly decreased when the medial forebrain bundle (MFB) was also severed. After lesioning only MFB a diminution of the three negative components was observed. The topical application of 6-hydroxidopamine did not modify the characteristics of the evoked potentials produced by posterior hypothalamic stimulation. In contrast the 3rd component of the potentials evoked by MFB stimulation first increased and then decreased. The 2nd component did not change with small doses (8; 14 μg) but decreased with higher doses (20; 40; 80 μg). The first component with lower doses decreased and with higher doses first increased and then decreased.

The results suggest that: a) these components of the evoked potentials result from the activation of different pathways; b) the activity of the MFB pathways see seem to be influenced by catecholamines.

224 GUSTATORY RESPONSES OF TWO SPECIES OF KANGAROO RAT. Kenneth J. Harper, James G. Kenagy*†, and Bruce Oakley, Div. Biological Sciences, Univ. Mich., Ann Arbor, MI, 48109; † Scripps Inst. Oceanography, LaJolla, CA, 92037.

Gustatory nerve responses were compared for two closely related, morphologically similar desert rodents with different natural diets. Both species occur in the same habitat but Dipodomys merriami feeds largely on seeds, whereas D. microps feeds extensively on saltbush leaves. This species comparison was intended to determine whether the natural diet plays a role in the evolution of taste receptor mechanisms. Integrated responses to taste solutions on the tongue were recorded from the intact chorda tympani nerve. The similarity of binding constants calculated from the neural responses to an NaCl (or sucrose) concentration series suggests that the observed species differences in NaCl (and sucrose) responsiveness may have been due to differences in the number of receptor sites but not to differences in affinity. There are two major species differences in the neural responses that can be related to diet. First, low concentrations of NaCl are more effective for D. merriami than for D. microps. Unlike the saltbush diet of D. microps the seed diet of D. merriami is NaCl deficient. Second, sucrose is more effective for D. merriami than for D. microps. One might expect from D. merriami's greater reliance upon the oxidation of carbohydrates as a source of water, that it would have a larger sucrose response. Sucrose is the most prevalent free sugar in seeds. We suggest that these species differences in gustatory physiology are adaptive evolutionary responses to differences in natural diet. Behavioral preference tests are presently being conducted.

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225 RECOVERY OF OLFACTORY BULB RESPONSE TO ELECTRICAL EXCITATION OF OLFACTORY NERVE IN LONG-NOSED GARFISH. Jeffrey A. Kugel and Dexter M. Easton. Dept. of Biol. Sci., FSU, Tallahassee, FL 32306.

A fluid electrode stimulation and recording system permitted electrical stimulation and recording of the full range of the olfactory nerve input to the olfactory bulb in the long-nosed garfish, Lepisosteus osseus. The nerve compound action potential, recorded en passage, served as a monitor of the level of activation of the nerve. The resulting evoked potentials were recorded from the bulbar surface by a pipette electrode. The recovery cycle of the bulbar response in a two-pulse, conditioning-testing experiment was about 500 times as long as the recovery cycle of the input nerve. The bulb required more than 15 seconds for recovery, whereas the nerve recovered within about 30 msec. Repetitive stimulation at maximum nerve response amplitude and frequencies up to 2/sec. resulted in the establishment of a depressed steady-state level of bulbar evoked response. However, stimulation at input levels less than maximum led to development of alternating-amplitude responses in the bulb. At lower input levels, the alternation developed at a lower frequency of stimulation. These observations are consistent with the presence of association systems within the fish olfactory bulb similar to those proposed for mammalian systems (Rall & Shepherd, 1968, J. Neurophysiol., 31, 884; Freeman, 1972, J. Neurophysiol., 35, 745, 762.)

(Aided in part by Psychobiology Program, Florida State University).

226 TURNOVER OF TASTE CELLS ON THE BARBELS OF CHANNEL CATFISH, ICTALURUS PUNCTATUS. Randie Raderman Little* (SPON: L.M. Beidler) Dept. Biol. Sci., Florida State University, Tallahassee, Florida 32306

There are many similarities between the taste receptors of mammals and some lower vertebrates. Renewal of taste cells has been demonstrated previously in mammals (Beidler and Smallman, 1965; Conger and Wells, 1969). The life span was shown to be approximately 10 days. I have determined that taste cells are also renewed in catfish.

Channel catfish, 8 to 12 cm in length and held in 20°-22°C water were injected intraperitoneally with 3 µc/gm body weight of ^{3}M thymidine. Barbels were sampled between day 2 and day 22 after injection. Tissue fixed in aqueous Bouins, dehydrated and embedded in paraffin was cut in 5 µm sections and prepared for autoradiography. The number of labeled cells per center cross section of taste bud were counted at various times after ^{3}H thymidine injection. Results show that epithelial cells surrounding the taste buds in the barbel divide and some of their daughter cells migrate into the the taste buds. At early sampling times (2 days) many labeled cells can be seen surrounding the taste buds but cells within the bud are rarely labeled. At later times (8 to 10 days) many taste cells are labeled. After 8 to 12 days the number of labeled cells inside the taste bud decreases. Preliminary results indicate that the turnover time or life span of the taste cells in on the order of 12 - 16 days. Current work on the effect of temperature on turnover and the effect of low temperature on taste bud structure will also be discussed. (Supported by NIH grant NS05258).

227 SEPARTE OLFACTORY AND NEURAL SYSTEMS MEDIATE MATERNAL BEHAVIOR, CANNIBALISM, AND CRICKET KILLING IN THE FEMALE HAMSTER. David M. Marques & Elliot S. Valenstein. Psych. Dept. and Neuroscience Lab., University of Michigan, Ann Arbor, Michigan, 48109.

When virgin female hamsters are exposed to 2-5 day old pups, approximately 60% of them carry the pups to their nest while the remaining females kill and cannibalize them. These response patterns are displayed after less than 2 min of exposure and are consistent across repeated testing. The present experiments were designed to investigate the contributions of peripheral olfactory structures to these response patterns.

Radical bilateral olfactory bulbectomy intended to include destruction of the anterior olfactory nucleus (AON) decreases both carrying and killing in virgin females. This is not due to anosmia since olfactory mucosa destruction by zinc sulfate (ZS) by itself has no significant effect on the prevailing response pattern. More conservative, rostral bulbectomy intended to spare the AON converts "carriers" into "killers," while cutting the vomeronasal nerves (VNNC) tends to convert "killers" into "carriers." Adding ZS treatment to the VNNC animals converts the remaining "killers" into "carriers."

These effects are not due to changes in general aggressiveness as none of the treatments reduces cricket killing. All intact and operated hamsters killed crickets in our tests. Nest building and food hoarding, on the other hand, are eliminated by both radical and conservative bulbectomies, but by no other treatment. It appears that the accessory olfactory system and the AON contribute to pup-killing. The main olfactory system (but not smell) is necessary for maintaining pup-carrying and for nest building. Neither system is important for cricket killing. These two olfactory systems are known to have separate central projections. Thus the neural systems mediating cannibalism, maternal behavior, and cricket killing in the female hamster appear to be separate.

228 EFFERENT CONTROL OF STIMULUS ACCESS TO THE VOMERONASAL ORGAN (VNO). Michael Meredith* (SPON: C. Pfaffmann). Rockefeller Univ. New York, NY 10021.

Possibly important in mating behavior (Powers & Winans, Science, 187: 961, 1975), the hamster VNO is closely associated with cavernous vascular tissue innervated by the nasopalatine (sphenopalatine) nerves. Stimulation of these nerves confirms a long-standing speculation (Herzfield, 1882) that vasoconstriction and dilation can pump fluids in and out of the VNO lumen. Nasopalatine (NP) nerve stimulation produces flow through the VN pore, correlated both with expansion and collapse of the side wall of the organ (overlying the cavernous tissue) and with changes in firing frequency of accessory olfactory bulb units (innervated by VN afferent fibers). Low level NP stimulation ($\pm$ 10 pulses @ 20-50μA) produces a biphasic vasomotor response of approximately constant time course. Amplitude of response depends on number of pulses and current. After <1 sec latency (for 4-6mm conduction distance) VN vessels dilate briefly (approx. 1 sec) then contract, reaching maximum contraction approximately 3 sec after stimulation and returning gradually to baseline after approximately 30 sec.

Sympathectomy, sympathetic trunk stimulation and intracarotid epinephrine injection suggest that the contraction is mediated by adrenergic fibers from the superior sympathetic ganglion. The initial dilation remains when the contraction disappears after superior cervical sympathectomy (and degeneration of postganglionic fibers) and appears to have a lower threshold and a different sensitivity to interstimulus interval within a stimulus train. Dilation may involve a separately innervated system which would empty the luminal contents.

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229 SPATIAL SUMMATION AND LATERAL INTERACTION IN PERIPHERAL GUSTATORY NEURONS. Inglis J. Miller, Jr. Department of Anatomy, Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, N. C. 27103.

Single chorda tympani neurons in the rat receive gustatory input from one or more taste buds located in different fungiform papillae. The peripheral afferents branch in the tongue so that response spike trains recorded in proximal regions of chorda tympani fibers result from the summation of multiple simultaneous inputs. Responses were recorded from single chorda tympani fibers when single fungiform papillae or the entire tongue was stimulated with NaCl. Mean responses from five single fibers in response to NaCl concentrations of 0.1, 0.3, and 1.0m were 26, 51, and 68 impulses/4 for stimulation of single papillae, while stimulation of the entire tongue evoked mean responses of 38, 72, and 94 impulses/4 sec with the same stimuli. In this series of trials the single papillae accounted for about 70% of the fiber response to stimulation of the entire tongue. With water applied to the single papillae and NaCl applied to the region surrounding the isolated papilla, the mean responses of the five units were 25, 50, and 57 impulses/4 sec or about 70% of the entire tongue response. Thus, stimulation of the entire tongue results in a loss of about 30 - 40% of spike activity which can be generated by independent stimulation of the single papillae, and the loss occurs at response levels of only 6 - 7 spikes/sec. Because this lateral depression occurs at such low response frequencies, spike collision probably contributes minimally to a mediation of the effect. A more pausible explanation suggests that some region of the terminal arborization of branched gustatory afferents has a diminished safety factor to impulse generation or transmission which is contingent on the activity in collateral branches.

(Supported in part by NIH Grant NS 10389.)

230 PERCEIVED INTENSITY OF ODOR-TASTE MIXTURES. Claire Murphy*, Linda M. Bartoshuk and William S. Cain*. John B. Pierce Fndn. Lab., 290 Congress Ave., New Haven, CT 06519.

Subjects estimated the intensity of various concentrations of an odorant (ethyl butyrate), a tastant (sodium saccharin), and mixtures of the two. The question of interest was whether the perceived intensity of the mixture would be equal to, greater than, or less than the intensities of the unmixed components. In fact, the two systems showed very close to simple additivity: the intensity of the mixtures was very close to the sum of the perceived intensities of the unmixed components. When odor and taste were examined separately another effect emerged. Subjects ascribed little or no odor to solutions containing only sodium saccharin but ascribed a considerable taste to solutions containing only ethyl butyrate. The taste ascribed to ethyl butyrate was not due exclusively to its action on the taste system since when the nostrils were closed as much as 80% of this "taste" disappeared. Hence, when there is simultaneous input to both the olfactory and taste systems, subjects resolve ambiguity regarding the locus of the stimulation in favor of the taste system.

231 RESPONSE PLASTICITY IN HAMSTER OLFACTORY BULB: PERIPHERAL AND CENTRAL PROCESSES: Harry Potter* and Stephan L. Chorover, Dept. of Psychology, MIT, Cambridge, Mass. 02139.

Odor responses observed in both the receptor and in mitral cells within the olfactory bulb (OB) are known to decrease upon repeated or prolonged presentation of the stimulus. The receptor response, as measured by the electroolfactogram (EOG), diminishes during continuous odor stimulation, but rebounds to full strength within 60 to 120 seconds after removal of the odor. On the other hand, mitral cell responses continue to decline even with interstimulus intervals as great as 5 minutes, and total recovery of original response levels requires 15-30 minutes. In addition some mitral cells exhibit longterm shifts in response character. These data suggest that mitral cells exhibit true habituation and may be worth studying as a model of physiological response plasticity.

The possibility that centrifugal inputs to the OB play a role in this plasticity led us to examine mitral cell responses following the disconnection of the OB either from the anterior olfactory nucleus (AON) alone or from all centrifugal inputs. Odor responses among mitral cells in such preparations habituated much more rapidly and took longer to recover than in the intact preparation. Also these cells exhibited faster firing rates, hyperexcitability, and a lack of highly tuned synchronization with inhalation. Reconstruction of the lesions suggests that the loss of AON input alone could be responsible for all of these effects.

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232 RAPID TRANSNEURONAL DEGENERATION IN THE PIRIFORM CORTEX OF THE RAT. J. L. Price. Dept. of Anat. and Neurobiol., Washington Univ. Sch.of Med. St. Louis, Mo. 63110

Autoradiographic experiments with injections of ^{3}H-Leucine into the olfactory bulb have shown that the termination of fibers to the piriform cortex is sharply restricted to the outer part of layer I (IA) (Price, 1973). However, in material prepared by the Nauta or Fink-Heimer methods 4 to 5 days after lesions of the olfactory bulb, axonal and terminal degeneration is consistently present in the deeper layers of the cortex, in addition to the dense degeneration seen in layer IA. In the latter material argyrophilic neurons are also found in the most superficial part of layer II (e.g. Heimer, 1968). Recent observations indicate that these cells are undergoing transneuronal degeneration, and may account for the axonal degeneration in the deeper cortical layers.

Experiments with injections of horseradish peroxidase into the olfactory bulb and cortex have shown that the pyramidal cells in the outer part of layer II form a separate sublayer (IIa) with projections to the anterior olfactory nucleus, olfactory tubercle, and lateral entorhinal cortex which are distinct from the projections of cells in other layers. The cells of layer IIa do not project directly to the olfactory bulb, although cells of layers IIb and III project to the granule cell layer of the bulb (Haberly and Price, 1976). Two weeks following olfactory bulb ablation in adult rats, the neurons of layer IIa show marked shrinkage. With longer survival times (8 to 15 weeks) these cells can no longer be recognized in Nissl material; layer IIa has apparently disappeared and layer II as a whole is substantially thinner than on the contralateral, normal side. Preliminary electron microscopical examination indicates that darkened, apparently degenerating dendrites and cell somata are found in the piriform cortex after an olfactory bulb lesion.

233 Coding of Taste Intensity in the Thalamus of the Rat. Thomas R. Scott and Michael S. Yalowitz.* Dept. Psychol., Univ. of Delaware, Newark, DE 19711

Patterning theories of taste coding hold that stimulus quality is represented by the shape of the evoked neural response envelope while intensity is carried by the total activity under the envelope. Most studies at the chorda tympani and solitary nucleus levels support these contentions. But lower-order taste neurons give uncomplicated responses which are typically excitatory and of short latency. Thalamic (fourth-order) neurons, by contrast, show greater variability in response characteristics, and thus the coding of taste intensity may be more convoluted. Male albino rats, Flaxedilized and locally anesthetized, were chemically stimulated with a variety of taste qualities, each at three different intensities. Tungsten microelectrodes recorded evoked single neuron activity from 39 cells in the gustatory region of the ventrobasal complex. While summed neural activity tended upward with greater stimulus intensity, the increases were neither monotonic nor universal across stimuli. Some individual neurons showed progressively greater excitation at each higher intensity of all stimuli, but others consistently decreased their activity. Most responded in a more complex fashion, the response to changing intensity depending on stimulus quality. Occasionally the intermediate intensity would cause maximal excitation, with lower evoked activity to more and less concentrated solutions. The opposite intensity-response function (lowest response to the intermediate concentration) was also noted. Many stimuli inhibited spontaneous neural activity, to a degree dependent on a solution's intensity as well as quality. The coding of taste stimulus intensity in the thalamus appears as complex as intensity coding in the visual and auditory systems. These results do not fit within the framework of patterning theories of gustatory neural coding, as presently formulated.

234 THE ROLE OF OLFACTION IN THE NUTRITION OF RAT NEONATE. Farhad Shafa, Esmail Meisami, Robabeh Moosavi. Dept. of Cell&Molecular Biol. Fac. Science. Tehran Univ., P.O. Box 14-1700 Tehran, IRAN.

Factors which aid the rat neonate to locate the mothers nipple prior to eye-opening are not well understood. It is reasonable, however, to count: maternal aiding, littermate interactions, tactile, gustatory and olfactory cues as possible factors. Olfactory cues are mediated through the olfactory bulb which in spite of posessing almost mature mitral cells at birth, lacks much of the glomerular organization which appears later in life. To explore the capability of the rudimentary olfactory structures of the neonate in discriminating olfactory cues necessary for its nutrition, we rendered them anosmic at 3 days of age by two methods. In one we compared bilaterally bulbotomised(BB) rats with their monolaterally bulbotomized (MB) and sham operated littermates. The body wt. of BB vs. MB littermates was lower by 32%, 21%, 20% and the brain wt. was lower by 15%, 9%, 11% at 10, 15, and 25 days of age respectively. MB animals showed a slight reduction in these weights compared with sham operated littermates. In another experiment the olfactory mucosa was destroyed by nasal injection of 3% $ZnSo_4$ Soln. These were compared with littermates which were injected with saline in the nose and $ZnSo_4$ in the stomach. The body wt. of anosmic littermates was lower by 31%, 31%, 26% at 10, 15 and 25 days of age respectively, and brain wt. was lower by 12% at day 25. In both experiments survival of anosmic animals was lower by 20% compared with their respective controles. These results indicate that anosmia in the rat neonate results in death or reduced body and brain growth, and suggests further that despite its rudimentary form prior to weaning, the olfactory bulb posesses the essencial structures necessary for guiding the young to find the mothers nipple through olfactory cues. Supported by Tehran Univ. &Ministry of Sci. IRAN.

235 UNDERWATER ELECTRO-OLFACTOGRAM RECORDINGS FROM THE ATLANTIC STINGRAY (DASYATIS SABINA). Wayne L. Silver* (Spon: B.W. Robinson). Dept. of Biological Science, Florida State University, Tallahassee, Florida 32306.

Little information is available concerning the sense of smell in marine fishes. Attempts to record surface neural responses (A.C.) from olfactory receptors, as has been done in freshwater fishes (Sutterlin, A.M. and Sutterlin, N., J. Fish. Res. Bd. Can., 28:565, 1971; Suzuki, N. and Tucker, D., Comp. Biochem. Physiol., 40A:399, 1971), were unsuccessful due to shunting of the electrical signals by the highly conductive sea water. However, it was possible to study olfactory responses by recording the larger signal-to-noise-ratio D.C. potentials. The EOG responses were recorded in vivo with calomel electrodes via Ringer-agar filled capillary pipettes. Amino acids, shown to be highly stimulatory compounds in freshwater fishes (see refs, above) were used as stimuli. Of the 14 amino acids tested on the stingray, the five most effective were L-glutamic γ methyl ester > L-ethionine > L-serine > L-glutamic acid > L-methionine. The L-isomer of an amino acid was more effective than the D-isomer. The average response magnitude for the more effective amino acids at 10^{-3}M was 0.2 mV. The response increased exponentially with logarithmic increase (spanning 4-5 log units) of concentration. Threshold concentrations for the more effective amino acids ranged between 10^{-6}M and 10^{-8}M. (Supported by NIH grants, NS-08814 and NS-05258.)

236 OLFACTORY CUES IN NIPPLE ORIENTATION AND ATTACHMENT IN RAT PUPS. Pauline J. Singh and Myron A. Hofer. Dept. Psychiatry, Albert Einstein Col. Med., Montefiore Hosp., Bronx, N.Y. 10467.

Central or peripheral olfactory deafferentation severely disrupts nursing in rat pups,but this may be the result of a performance deficit as well as a sensory deficit. In this experiment, pups were left intact, but the olfactory properties of the mother's ventrum were altered. Fifty-two litters (8 to 9 days old) were observed, each litter having been culled to 7 pups. Each pup was placed on the ventrum of its anesthetized mother and total number of pups that attached to a nipple within a three-minute period were recorded. It was found that 94% of the pups attached to a nipple of their untreated mother; but after washing the ventrum with acetone and alcohol, rinsing with water, and blow-drying (shampoo) only 21% attached. Control experiments indicated that this decrease in attachment was not due to repeated testing, lowered ventral skin temperature, or aversion to traces of solvents on the ventrum. Cow's milk applied to the nipples of a shampooed mother did not increase attachment, whereas applying a substance(s) suctioned from the nipple area did if the donor mother was on the same diet and did not if she was on a different diet. If a shampooed mother was injected IP with oxytocin (10 units/kg), attachment increased from 17% (pre-oxytocin) to 79% (post-oxytocin). This occurred even when the nipples were ligated (19% pre-oxytocin to 76% post-oxytocin). Results suggest that some substance(s) in the nipple area other than milk is emitted in response to circulating oxytocin and acts as an olfactory cue for the pups. Perhaps it is secreted from the apocrine Montgomery glands which may have the function of a scent organ.

237 SOURCES OF OLFACTORY PROJECTIONS TO THE DORSAL THALAMUS IN THE TREE SHREW (Tupaia glis). L. C. Skeen, Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710.

Previous experiments using the anterograde transport of tritiated amino acids have demonstrated that the olfactory bulb in Tupaia projects to layer Ia of several paleocortical regions and that these regions project, in turn, to the medial dorsal nucleus of the thalamus (MD) and, hence, to prefrontal neocortex (Skeen, 1974; 1975). As a further step in an analysis of the relationships between the olfactory bulb and prefrontal neocortex, the present experiments were aimed at identifying the neurons which participate in these pathways. The distribution of the paleocortical neurons which receive direct olfactory projections was analyzed using the anterograde transport of TCA-insoluble tritiated nucleoside derivatives to postsynaptic elements (Schubert and Kreutzberg, 1974; 1975) following injections into the olfactory bulb (0.25 µl/250µCi, ^{3}H-Adenosine). The distribution of paleocortical neurons which project directly to MD was analyzed using the retrograde transport of horseradish peroxidase following injections into MD (0.1 µl/30% HRP, Sigma type VI). Results obtained so far indicate that one population of neurons in the olfactory paleocortex, large neurons in the polymorphic cell layer III of the pyriform cortex, receive direct olfactory projections and project to MD. Further analysis will be aimed at determining whether the neurons in this population which receive direct olfactory projections are the same neurons which terminate in MD. (Supported by NSF Grant No. 75-04230.)

238 SPECIFICITY IN OLFACTORY DEVELOPMENT: PHARMACOLOGICAL EVIDENCE. Sonya K. Sobrian and Catherine Cornwell-Jones*. Dept. Psych., Princeton University, Princeton, New Jersey, 08540.

The odor preferences and brain catecholamine content of Sprague-Dawley rat pups 5-16 days of age were selectively altered following neonatal subcutaneous injections of 50 g/g of 6-hydroxydopamine on days 0-3 postpartum. When tested in a 2-choice situation preventing tactile or gustatory cues and requiring a locomotor response, 6-OHDA treated pups 5-7 days old were indifferent to the odor of their own nest shavings in contrast to saline-injected controls who were strongly attracted by this odor. However, between 8 and 16 days of age, preferences for nest odor were similar in both groups. The early 6-OHDA-induced deficits were indicative of sensory rather than locomotor differences in that lemon odor repelled control and drug-treated pups equally. Differences in behavioral development coincided with changes in catecholamine content in various olfactory regions of the brain. 6-OHDA decreased NE by 54% in the olfactory bulb of 5 but not 8 or 13 day old pups. DA levels in the olfactory tubercles were not altered between 5 and 16 days of age following 6-OHDA. These results suggest a specificity in the neurological substrates mediating the development of behavior guided by the two test odors. 6-OHDA treatment disrupts the substrates mediating responses to nest odor but not lemon. These are the first data indicating that systemic neonatal systemic neonatal 6-OHDA treatment alters sensory development.

239 A DIRECT OLFACTORY PROJECTION TO AREA FRONTALIS IN THE OPOSSUM. R.C. Switzer and L. Heimer. Dept. Anat., Sch. Med., Univ. Va., Charlottesville, Va. 22901.

The primary olfactory projections of the opossum were studied with de Olmos' cupric silver method following unilateral removal of the olfactory bulb. The lesions did not include the accessory olfactory formation or the anterior olfactory nucleus. Degeneration was observed, not only in paleocortex and olfactory tubercle, but also in the frontal neocortex dorsal to the rhinal sulcus (area frontalis of Gray). In accordance with the results obtained by Scalia (JCN 161:31, 1975) degenerating fibers were seen in neocortex following survival times of 3-8 days. Terminal degeneration in neocortex, however, was seen only in animals with 2 days survival time. The neocortical olfactory projection area in the opossum seems to be in close proximity to the somatosensory and motor representation for tongue, lips and intraoral areas (Pubols et al., JCN 165:229, 1976).

A reexamination of the olfactory projections in the rat has revealed a similar but less dramatic projection of olfactory bulb efferents to the so-called sulcal cortex on the dorsal lip of the rhinal sulcus. This juxtallocortical olfactory projection area in the rat seems to be, if not identical with, at least in immediate proximity to the cortical taste area demonstrated by Benjamin and Akert (JCN 111:231, 1959). Considering the very close relationship that exists between olfaction and taste behaviorally, this convergence of thalamocortical gustatory projections and primary olfactory bulb projections to nearby, or maybe even partly overlapping cortical regions is hardly surprising.
(Supported by NIH Grants NS02558 and NSI0972)

240 RESPONSE PROPERTIES OF TASTE NEURONS IN THE NUCLEUS TRACTUS SOLITARIUS OF THE HAMSTER. Joseph B. Travers* and David V. Smith. Dept. Psychol., Univ. Wyoming, Laramie, Wyo. 82071.

The responses of hamster chorda tympani neurons have been classified as "sucrose-best", "NaCl-best", or "HCl-best", depending upon their relative sensitivities to these basic taste compounds (Frank, M., J. Gen. Physiol., 61:588, 1973). This analysis has led to the suggestion that taste quality is coded by activity in specific "labelled" neurons, e.g., sweetness by activity in "sucrose-best" units. Recordings were obtained from individual neurons in the nucleus tractus solitarius (NTS) of anesthetized hamsters to stimulation of the anterior portion of the tongue with 0.03 M NaCl, 0.1 M sucrose, 0.003 M HCl, and 0.001 M quinine hydrochloride. Response profiles to these four basic taste stimuli were derived for each unit in a manner similar to those reported for hamster chorda tympani fibers. Although units in the chorda tympani are quite strikingly specific in their responsiveness to these compounds, NTS neurons are much more broadly tuned. Identification of units as clearly "sucrose-best", "NaCl-best", or "HCl-best" was virtually impossible, as most of the NTS neurons tended to respond vigorously to two or three of these compounds. As in the hamster chorda tympani, quinine hydrochloride was the least effective stimulus for these neurons, and in several units quinine actually reduced the impulse frequency below that produced by flowing distilled H_2O over the tongue. Compared to units in the rat NTS, hamster NTS neurons do not show an increase in impulse frequency over that of chorda tympani fibers. The relative lack of specificity of neurons in the hamster NTS to these basic taste compounds raises some question as to the viability of a "labelled-line" approach to understanding gustatory quality coding in the central nervous system. (Supported by NINDS Grant NS10211 and Research Career Development Award NS00168).

241 NASAL TRIGEMINAL RESPONSES IN THE ROSTRAL MEDULLA. Richard L. Van Buskirk* and Robert P. Erickson. Dept. of Psych., Duke University, Durham, N.C. 27706.

Trigeminal afferents from the nasal mucosa have been shown to be odor responsive and appear to be involved in odor discrimination (Tucker, 1971). Electrical stimulation of nasal trigeminal afferents elicit neural responses in the caudal trigeminal nucleus and neighboring reticular formation, but no responses to odorant stimulation have been evoked in these neurons (Beuerman, 1975). Our study was to determine the extent to which odorant-evoked responses could be identified in the areas of first-order trigeminal terminations, with particular attention to the possibility of odorant-taste interactions. The ethmoid nerve, a nasal trigeminal branch, was stimulated electrically in the rat, and responses recorded with micropipettes in the rostral medulla. Responsive neurons were found in trigeminal n. oralis, in the medially adjacent n. reticularis parvicellularis and dorsally within the gustatory nucleus of the solitary tract. These units displayed a wide range of response latencies (2 to 180 msec) with a mean latency for all units of 14.1 msec. About 39% of those units found in dorsal n. oralis and medially into NTS (mean latency 21 msec) also responded to mechanical or chemical stimulation of the tongue. Units in the ventral 2/3's of n. oralis and adjacent reticular formation did not display tongue receptive fields. Seventeen of 56 gustatory neurons showed slow developing responses to the odorant methyl alcohol and the odor of rotten rat chow. Labile responses were obtained to acetone, benzaldehyde, and amyl acetate at concentrations known effective for the ethmoid nerve. (Supported by NSF grant BNS 75-22692)

242 ELECTROANTENNOGRAMS AND UNIT RESPONSES OF DROSOPHILA OLFACTORY RECEPTORS J. A. Weier* and R. C. Gesteland, Dept. Biol. Sci., Northwestern Univ., Evanston, IL., 60201.

A possible approach to the determination of chemical mechanisms for olfactory reception is to study mutants deficient in their capacity to respond to particular stimuli. *Drosophila melanogaster* is well suited for such studies. First it is necessary to characterize the properties of the summated receptor current (the electroantennogram or EAG) and the responses of single receptor neurons in the wild type to a wide vareity of stimulus substances. EAGs vary in shape from simple, antenna-tip negative potentials to complex polyphasic responses, depending upon the stimulus substance. Response magnitude as a function of vapor concentration and the slope of the log-log plot of response amplitude *vs.* concentration were determined for homologous series of alcohols, carboxylic acids, aldehydes, ketones and ethers and a variety of armoatic and heterocyclic compounds. Concentrations required to evoke equal amplitude responses differed for different substances, as did slopes. Single neurons differed in their responses to any particular substance, both in number of evoked spikes and latency. Any cell responded to many stimuli. Vapors of natural products important to the fly tended to be more effective in exciting the cells than did pure organic compounds in our stimulus set. That cells are different in their sensitivity to particular stimuli was clearly shown in some preparations where the EAG electrode recorded the spike activity from a small population of cells simultaneously with a microelectrode recording from a single cell. We do not yet know if cells with different response properties can be identified with morphologically distinguishable receptor sensilla types. Supported in part by NSF Grant No. BMS75-02339.

243 NEUROPHYSIOLOGICAL RESPONSES FROM CHEMOSENSORY UNITS OF CAT AND DOG GENICULATE GANGLION TO CHEMICAL STIMULATION OF THE TONGUE. T. D. White* and James C. Boudreau. (Sponsor: H. G. Sperling. Sensory Sciences Center, University of Texas, Graduate School of Biomedical Sciences, Houston, Texas 77030.

Three functional classes of chemosensory neurons have been identified in both the cat and dog geniculate ganglion with single unit neurophysiological recordings. An across species comparison was made between the responses elicited from Group I and Group II neural units by a variety of chemical stimuli including amino acids, nucleotides, and inorganic salts. The optimum stimuli for Group I units of both species were identical (malic acid, HCl, ATP, ITP, and L-histidine). L-proline and L-cysteine were maximally stimulatory for Group II units of both species, although the cat units were more responsive to ITP, IDP, ATP, HCl, and L-histidine. Group II units of both animals also exhibited an increase in response to an increase concentration of a NaCl solution. Group I units were considerably less sensitive to NaCl and only the cat units exhibited an increase in response to an increase in concentration (> 1M). Further comparison of Group II units' responses to amino acids demonstrated that the cat units discharged more to hydroxy (serine and threonine) and acidic (glutamic and aspartic) amino acids. The responses of Group II units from both carnivores to a set of nitrogen heterocycles were also similar (r=.77) with pyrrolidine, 3-pyrroline, and L-2-azetidine carboxylic acid being highly stimulatory. Supported in part by NSF Research Grant.

244 SOME RESPONSE PROPERTIES OF DEEP SHORT AXON CELLS IN THE OLFACTORY BULB OF THE SYRIAN GOLDEN HAMSTER. William M. Youngs*, Stephen Schneider* and Foteos Macrides. The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Response properties of "deep" short axon (SA) cells in the olfactory bulb (OB) were studied following electrical stimulation of the contralateral OB and the ipsilateral olfactory mucosa (OM) and lateral olfactory tract (LOT). Approximately one third of the deep SA cells in our sample showed excitatory responses to trains of biphasic square wave pulses applied to the contralateral OB. Typically, the mean number of spikes in an excitatory response increased monotonically with the train duration. Short axon cells which were excited by contralateral OB stimulation had significantly longer latencies to LOT and OM stimulation than any other class of spike generating cells recorded in the OB. Appropriately timed paired stimulations revealed that all excitatory responses were followed by a proportional period of reduced excitability. Anatomical and electrophysiological evidence suggest that the anterior limb of the anterior commissure, which contains axons from cells in the anterior olfactory nuclei (AON), provides a pathway for interbulbar effects. We postulate that cells in the AON comprise part of a centrifugal excitatory pathway onto deep SA cells. A possible mechanism for the disinhibition of bulbar output neuron activity involving centrifugal activation of deep SA cells will be discussed.

Comparative Neurobiology

245 ASCENDING EFFERENT TECTAL PROJECTIONS IN THE LIZARD GEKKO gecko (L.). Ann B. Butler. Dept. Anat., Georgetown Univ., Washington, D.C. 20007.

Eleven adult geckos underwent unilateral (9 cases) or bilateral (2 cases) aspiration lesions of the optic tectum under sodium pentobarbital anesthesia. After postoperative survival times of 12-16 days, the animals were sacrificed by transcardial perfusion of 10% formalin. The brains were frozen, sectioned at 30u, and processed with the Eager ('70) silver impregnation method for anterograde degeneration. The pretectal nuclei described in Iguana (Butler and Northcutt, '73) can all be recognized in Gekko, and the tectum projects bilaterally to five of these nuclei: geniculatus pretectalis (NGP), lentiformis mesencephali (NLM), posterodorsalis (NPD), dorsalis pretectalis (NDP), and ventralis pretectalis (NVP), but not to nuclei medialis pretectalis or lentiformis thalami. The projection to NPD bilaterally is quite sparse, and contralateral projections to NGP, NVP, NLM, and NDP are less dense than on the ipsilateral side.

In the thalamus, the tectum projects bilaterally to nuclei geniculatus lateralis pars ventralis (NGLV), rotundus (R), and sparsely to geniculatus lateralis pars dorsalis (NGLD). Projections to the contralateral thalamus pass rostrally through the supraoptic decussation and are less dense than on the ipsilateral side. The pattern of degeneration in nucleus rotundus bilaterally, following unilateral tectal lesions, is not uniformly distributed within the nucleus, but is arranged in a net-like, reticulated pattern of short bands with seemingly random orientation, as seen in both transverse and sagittal sections. These bands are not aligned with the fascicles of fibers entering the nucleus, and gaps between the bands are free of argyrophilic debris. Following bilateral tectal lesions, nucleus rotundus is entirely filled with degeneration. These results suggest that there is some form of segregation of the ipsilateral and contralateral tectal inputs along dendrites of rotundal neurons.

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246 BEHAVIORAL EFFECTS OF ELECTRICAL STIMULATION IN THE TELENCEPHALIC LOBES OF FREE-SWIMMING NURSE SHARKS, GINGLYMOSTOMA CIRRATUM. Leo S. Demski. Dept. Anat., Louisiana State Univ. Med. Ctr., New Orleans, LA. 70119 and The Gulf Coast Research Laboratory, Ocean Springs, MS. 39564.

Techniques were developed for chronic implantation of monopolar electrodes in the brains of juvenile nurse sharks. In this initial survey, over 30 electrodes were placed in scattered regions, mostly in the central and caudal portions of the telencephalic lobes in 9 animals. Sharks were stimulated in large rectangular tanks containing artificial sea-water, a gravel substrate and in most cases other nurse sharks and several types of frozen foods. Techniques, previously described in detail (Demski and Gerald, Brain Beh. Evol., 9, 41, 1974) were used to test and histologically identify stimulation sites. A maximum current of 1.5 MA was used and failure to elicit a response after 60 sec of stimulation at 5 and 50 Hz was considered a negative response. All negatives were followed by positive responses evoked from other sites in the same animal.

Strong "Arousal-Escape" was evoked from several sites in the caudodorsal regions of the telencephalic lobe. This area includes Holmgren's primordium hippocampus and adjacent portions of the central nucleus (terminology of Schroeder and Ebbesson, Brain Beh. Evol., 9, 121, 1974). Stimulation sites rostral, caudal and dorsal to this area were negative for this behavior. Five Hz stimulation was much more effective than 50 Hz stimulation in eliciting this response (higher frequencies seemed to inhibit the response). "Arousal-Escape" usually consisted of the following pattern; rapid swimming near the surface, lifting the head out of the water, climbing over a Plexiglas tank divider that extended 4 to 6 inches above the surface of the water and frequent attempts to scale the tank walls. This response began slowly and seemed to increase in intensity as the stimulation was continued. The activity did not wane and there was a post-stimulus facilitory period during which it could be re-evoked with much shorter latency. Thresholds ranged from .7 to 1 MA. Based on similarities in the responses evoked by electrical stimulation, this area in the caudodorsal region of the nurse shark telencephalon could represent the elasmobranch counterpart of the tetrapod amygdaloid complex.

Various combinations of spitting or coughing, head shaking, barbel movement, eye retraction and backing up on the folded pectoral fins were evoked from several sites in the more rostral portion of the central nucleus (area near the rostral limit of termination of thalamic afferents according to Schroeder and Ebbesson, Ibid). Thresholds ranged from .2 to 1.5 MA with 50 Hz stimulation at least as effective as 5 Hz pulses. The type of activity evoked from this area suggests that it may play a role in sensorimotor control of peripheral regions within the trigeminal distribution.

Other responses evoked from the telecephalic lobes include: swimming, possible weak feeding and ipsiversive circling. The number of positive sites is insufficient to permit analysis of specific anatomical substrates for these behavior patterns. Completely negative sites were located primarily in the caudal part of the central nucleus and posterior pole of the lobe. Supported in part by Air Force Office of Scientific Research Contract 75-2803.

247 IONIC CONTRIBUTIONS TO THE RESTING AND ACTION POTENTIALS OF MAMMALIAN PERIPHERAL SENSORY NEURONS. Richard A. Jaffe and Sanford R. Sampson. Dept. Biol., Battelle-Northwest, Richland, WA. 99352 and Dept. Physiol., UCSF, San Francisco, CA. 94143.

The role of various ions in the maintenance and generation of resting and action potentials in mammalian neurons has not been thoroughly characterized. We have, therefore, examined the effects of changes in extracellular K^+, Na^+ and Cl^- on intracellular potentials recorded from cell bodies of neurons in nodose ganglia removed from anesthetized cats, and maintained in vitro at 37°C. Ganglia were initially superfused with a solution approximating the ionic composition of cat extracellular fluid. The solution was equilibrated with 95% O_2 and 5% CO_2, and its pH, P_{CO_2} and P_{O_2} were measured periodically and maintained constant throughout the experiment. In order to study ionic contributions, special solutions of altered ionic composition were also used to superfuse the ganglia. Data on passive and active membrane properties were obtained from 5-10 neurons in the normal superfusate, then the ganglion was superfused with the experimental solution, data being obtained from 5-10 additional neurons after a minimum exposure time of 15 min. Finally, the ganglion was returned to normal solution where data from 3-5 more neurons were obtained. This procedure was repeated with several different ganglia for each experimental solution. The major findings were as follows: (1) the maximum slope of the relation between $[K^+]_o$ and resting membrane potential was 25 mV per 10-fold change in $[K^+]_o$; reduction of $[Na^+]_o$ from 150 mM to 34 mM (Na^+_o replacement by either TRIS, choline, or sucrose) consistently caused a slight but significant depolarization (3-4 mV) of resting membrane potential and a marked decrease in action potential amplitude; the replacement of Cl^-_o by either glutamate$^-$ or methylsulfate$^-$ caused a 30-50% reduction in membrane resistance, but was without effect on other measured properties. Increasing $[Cl^-]_o$ from 106 mM to 128 mM caused a significant 5 mV hyperpolarization and a reduction in membrane resistance averaging 35%. These findings cannot be readily explained by existing ionic models based on invertebrate nerve membranes. Supported by Pulmonary SCOR Grant HL14201 and NIH Grant GM00927.

248 DEMONSTRATION OF A SYNCYTIUM BY THE MIGRATION OF HORSE RADISH PEROXIDASE BETWEEN NEURONS IN THE PRAWN, MACROBRACHIUM ROSENBERGII. E. Macagno, and D.R. Friedländer*. Dept. Biological Sciences,Columbia University, New York, NY 10027.

The existence of syncytial neurons in various organisms has been proposed a number of times and in almost all cases later disproved. One interesting exception may be the strongly electrically-coupled S cells in the leech, which have been recently reported (Frank, Jansen and Rinvik, J. Comp. Neur. 159:1-14,1975) to be syncytial on the basis of procion dye migration, electron microscopy and degeneration experiments. These three techniques, however, do not prove conclusively that the S cells have a continuous cytoplasm, since (a) small molecules (such as cobalt chloride and procion yellow) are known to cross electrotonic junctions, (b) only complete serial sections at the EM level would show the existence or lack of junctions, and (c) some distal processes separated from the perikaryon are known to survive for long periods of time without degeneration. One possible way to demonstrate the continuity between the cytoplasms of cells is to inject into one neuron a macromolecular marker which is too large to cross electrotonic junctions and to determine whether it is freely exchanged by the cells. We wish to report here our observation of the migration of horse radish peroxidase (HRP) between giant motor neurons in the abdominal ganglia of the prawn Macrobrachium rosenbergii. The syncytial nature of these neurons in different species of prawn was originally proposed by Johnson (J. Comp. Neur. 36:323-373,1924) and later confirmed by Holmes (Philos. Trans. 231:293-311,1942) who analyzed optical serial sections. We have repeated the analysis on I µm serial sections of Macrobrachia ganglia using our computer reconstruction technique (Levinthal, Macagno and Tountas, Feder. Proceed. 33:2336-2340,1974)and have confirmed the prior observations. Both giant motoneurons send single large processes medially where they appear to fuse and form one large fiber that travels caudally. This single fiber bifurcates in the vicinity of the fourth root, sending large branches laterally and out the fourth roots. Synaptic input from the medial and lateral giant fibers occurs on the symmetric branches after the bifurcation. (The electrical coupling between these cells and the passage of cobalt from one cell to the other have been recently shown by Ochner, Parnas and Spira.)(personal communication). HRP was injected into the cell body of one motoneuron using beveled glass microelectrodes. We used both pressure injection and iontophoresis to inject the dye, since pressure injection sometimes causes cells and processes to burst. After allowing the marker to migrate for varying periods of time, the ganglia were fixed, reacted with substrate to form the opaque precipitate, dehydrated and cleared, and observed as whole mounts. The HRP was clearly seen to have migrated from the injected cell body to the point of fusion at the midline and then back into the paired cell body, forward along the single process to the bifurcation point and out both roots. No other cells and their processes were found to contain the marker. The same results were observed using both injection techniques.

We believe our observation that large molecules (m.w. 40,000) migrate from the site of injection throughout the cytoplasm of both giant motoneurons is a strong indication that the two cells do, in fact, form a syncytium, supporting the evidence from serial optical sections. Furthermore, the exchange of marker occurs only between this pair of cells, and not with others, including the giant fibers that presumably make electrotonic junctions with the giant motoneurons. This research was supported in part by a DuPont Young Faculty Grant and by PHS Grant NS-09821.

249 FIBER ORIGINS OF CERVICAL VAGUS NERVE IN RABBITS, CATS AND RHESUS MONKEYS: INJECTION OF HORSERADISH PEROXIDASE PROVIDES EVIDENCE FOR BILATERAL PROJECTIONS IN MONKEYS. T.W. Robertson*, J. Wallach, N. Schneiderman and P. Neumann*. Dept. of Psychology, Univ. of Miami, Coral Gables,Fla.33124

This study assesed the fiber origins of the right cervical vagus nerve in rabbits, cats and rhesus monkeys. A saturated solution of horseradish peroxidase (HRP Type VI, 10mg/100ul Ringer's solution) was injected via a semi-micropipette into the proximal end of the severed right cervical vagus nerve. After HRP administration all animals were maintained for 48 hours prior to being sacrificed for histochemical assay. Adult rabbits failed to exhibit HRP precipitate in the dorsal motor nucleus (DMN) or nucleus ambiguus. In contrast, extensive cell body labeling of the ipsilateral DMN and n. ambiguus was noted in 11 day old rabbits. Adult cats showed two types of HRP labeling: limited cell body labeling of the ipsilateral DMN and n. ambiguus and extensive bilateral synaptic labeling of the nucleus tracti spinalis trigemini caudalis, nucleus tractus solitarius, dorsal nucleus intercalatus and commissural nucleus. Adult rhesus monkeys showed bilateral cell body labeling in the DMN and n. ambiguus. Approximately 10 times as many cell bodies containing the HRP precipitate were found in the ipsilateral DMN as in the contralateral nucleus. In caudal portions of the DMN, contralateral cell body labeling was confined to medium and small cells in the dorso-medial portion of the nucleus. In more rostral areas, HRP positive cells were located only in the ventrolateral portion of the DMN. These cells were also of the medium and small type. The ipsilateral DMN exhibited HRP positive cells in all areas and all types of cells. Bilateral cell body labeling in the n. ambiguus occurred, but was sparse. This study revealed important differences in vagal fiber origins among three mammalian species: (1) Limited bilateral efferent fiber projections were noted in monkeys, but not in rabbits or cats; bilateral efferent projections for the vagus nerve have not been reported previously, (2) Adult cats exhibited both efferent and afferent labeling, where as rabbits and monkeys showed only efferent labeling, (3) HRP labeling was noted in adult monkeys and cats, but not in adult rabbits although extensive cell body labeling did occur in rabbit pups. (Supported by NSF grant# BMS75-10967.)

250 TELENCEPHALIC VISUAL RESPONSES IN THE LIZARD Gekko gecko. Michael L. Andry* and R. Glenn Northcutt. Div. Biol. Sci's., University of Michigan, Ann Arbor, MI, 48109.

Surface and depth recordings of telencephalic visual responses in Nembutal-anesthetized (25mg/kg, i.p.) Tokay geckos were obtained with Platinum-Irridium (75μ core) electrodes. Regions in both rostral and caudal telencephalon exhibited surface negative responses. Subsequent depth recordings yielded response component polarity inversions and associated multiple unit activity. The rostral telencephalic area exhibited multiple units at the surface, as well as deeper units associated with response component polarity inversions. On and/or off cluster activity occurred to ipsi-, contra-, and bilateral ocular stimulation. Caudal telencephalic surface evoked responses were comparable in latency and polarity to those reported by Gusel'nikov and Ya. Supin (1963). Multiple unit activity associated with these caudal telencephalic evoked responses was found with electrode penetrations at depths of 2-4 mm. Subsequent histological examination of the electrode tracks revealed that the multiple unit activity occurred at the level of the lateral geniculate nucleus. This thalamic generator source may account for the surface evoked cortical potentials recorded in the caudal telencephalon. (Supported by NIH Fellowship No. 1 F22 NS 02622, and NIH Grant No. 2 R01 NS 11006.)

251 CIRCADIAN VARIATIONS IN THE CONTENT OF NEURO-DEPRESSING HORMONE IN THE NERVOUS SYSTEM OF THE CRAYFISH. Hugo Aréchiga. Dept. of Physiol. Centro de Investigación, I.P.N., México 14, D.F.

Evidences from various sources suggest that circadian rhythmicity of the nervous system of crustaceans is modulated through a hormonal channel (Aréchiga, H. and Naylor, E. In Biorhythms in the Marine Environment P. J. De Coursey Ed. Univ. of South Carolina Press, 1976 pp. 1-16), and a neurodepressing hormone (NDH) has been detected in the nervous system of the crayfish. In the present experiments NDH activity was measured along the 24-hour cycle. Most of NDH (about 65%) in the nervous system of the crayfish Procambarus bouvieri is located in the sinus gland of the eyestalk. In crayfishes kept either under a natural illumination regime (LD) or in constant darkness (DD) for several days NDH activity was determined at different times of the 24-hour cycle. NDH was extracted from the eyestalk with acetone and chloroform. The extract was heated at 80°C during 5 min. and centrifugued at 90°C during 5 min. and the supernatant was passed successively through G-25 and G-10 Sephadex columns. In this way, NDH could be separated from other peptides present in the sinus gland. The activity was determined by the reduction of the spontaneous firing rate of an identified motorneuron in the group of superficial abdominal flexors in an isolated abdominal ganglion. Both under LD and DD, NDH activity was higher during the day-phase of the 24-hour cycle. The sensitivity of the motorneuron to NDH also varied as a function of time of day and of time after the excision of the ganglion, thus suggesting a gradual desensitization to NDH.

252 RETINAL PROJECTIONS IN THE PAINTED TURTLE, Chrysemys picta. Andrew H. Bass*. Div. Biol. Sci's., University of Michigan, Ann Arbor, MI, 48109.

Three painted turtles were injected intraocularly with (^{3}H) proline (60μCi). The brains were fixed by perfusion at 48 and 72 hours postoperatively. Mounted paraffin sections were coated with NTB-2 emulsion, stored at 4° C. for 28 days, developed and stained with cresyl violet. Retinal fibers were found to project to a number of discrete cell groups in the contralateral thalamus, pretectum, tectum, and tegmentum. Five primary retinal targets were identified in the thalamus: the ventral and dorsal divisions of the ventral lateral geniculate nucleus (GLV), a ventral division of nucleus ventrolateralis, an area dorsal to GLV termed pars lateralis of the nucleus dorsolateralis (DLpl), and an area dorsomedial to DLpl termed nucleus dorsocentralis (DC). Three cell groups in the pretectum were heavily labelled: nucleus lentiformis mesencephali, nucleus geniculatus pretectalis,and nucleus posterodorsalis (PD). Heavy grain counts were also seen over layers 8-14 of the tectum and nucleus opticus tegmenti. In addition, somewhat lighter accumulations of grains were noted over the following ipsilateral thalamic and pretectal groups: GLV, DLpl, and PD. While these findings confirm many of those reported in previous studies of retinal projections in turtles, they are evidence for a more widespread distribution of retinal projections. This work was done in collaboration with R. Glenn Northcutt. (Supported by NIH Grant No. 2 R01 NS 11006.)

253 TERMINATION OF TONIC ELECTRORECEPTORS IN LATERAL LINE LOBE OF MORMYRIDS. C. C. Bell and C. J. Russell, Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon 97210.

In electric fish of the family Mormyridae there are three types of electroreceptors--small, medium, and large. The small are also known as ampullary or tonic receptors. The medium and large receptors are sometimes called tuberous or phasic receptors. All lateral line afferents, including electroreceptors, terminate in the lateral line lobe (LLL). Some of the properties of the second order cells connected with large and medium receptors have been established by Zipser and Bennett (J. Neurophysiol.,in press). To identify elements related to tonic receptors, we recorded from LLL during long duration (1 sec) transcutaneous electrical stimuli. Stimulation of the posterior lateral line nerve itself allowed us to distinguish afferent fibers from second or third order cells. There appear to be two main classes of LLL cells related to tonic receptors: 1) those which are excited at short latency by the nerve and which respond to outside positive stimuli with an increase in discharge rate; 2) those inhibited by the nerve and whose discharge rate is decreased by outside positive stimuli. In each case outside negative stimuli have opposite effects. Discharge rate in the tonic receptors themselves is increased by outside positive and decreased by outside negative stimuli. LLL cells appear to have small receptive fields of a few square cms and there is as yet no evidence of opposite effects evoked from the surround. Tonic receptors terminate ventrally in LLL immediately lateral to the nucleus of the lateral line lobe. The second order cells are located just lateral to this site of afferent termination, i.e. in a restricted ventral region of the cortex of the lateral line lobe. Within this region both tonic receptor afferent terminals and the central cells which they influence are somatotopically arranged. (NIH NS06728 & NSF BMS73-0687-A01)

254 IMMUNOHISTOCHEMICAL LOCALIZATION OF A BRAIN PROTEIN METABOLICALLY LINKED WITH BEHAVIORAL ADAPTATION IN GOLDFISH. Larry I. Benowitz and Victor E. Shashoua. Harvard Med. Sch. and McLean Hosp., Belmont, MA 02178.

The rate of synthesis for 3 cytoplasmic brain proteins increases by up to 100% when goldfish master a vestibulomotor task (Shashoua, Brain Res., 1976). To determine which brain cells are active in producing these metabolic changes, we have now prepared antiserum specific to one of the 3 proteins (β, MW = 32,000) for use in the 2-layer immunohistofluorescent method (Hartman, J. Histochem. Cytochem., 1972). The β protein was first isolated, purified and characterized as a single product by electrophoretic migration and by amino acid end-group analysis. Injection of β into rabbits then produced a monospecific antiserum, as evidenced by immunodiffusion studies and by combining immunochemistry with double-labeling methods. Anatomical localization studies were next carried out using the rabbit anti-β serum.

The β protein was found to be a specific marker for a family of $\sim 10^4$ cells, 9 - 15 μm in diameter, located primarily in the ependyma. This zone, which in the goldfish comprises almost 1/4 the width of the brain at some levels, surrounds the ventricular surface and contains numerous cells of diverse types. β-positive elements are seen in the ependyma all the way from the spinal cord up through the forebrain, but are most heavily concentrated in those areas where certain special sensory integrational zones have undergone the greatest expansion: i.e., in the mesencephalic optic tectum/torus semicircularis and in the rhombencephalic vagal lobes (visceral integrational center). Thus, the enhanced activity in a group of paraventricular cells appears responsible for at least one of the specific protein changes that is associated with the goldfish's behavioral adaptation. (Supported by the Medical Foundation and NINCDS grant #NS 09407.)

255 ASYMMETRY OF HOMOLOGOUS MOTONEURON FREQUENCY IN DORSAL SUPERFICIAL MUSCLES IN ABDOMEN OF THE HERMIT CRAB, PAGURUS POLLICARUS. William D. Chapple. Regulatory Biology Section, Biological Sciences Group, University of Connecticut, Storrs, Ct. 06268

The relationship between tonic firing frequency and motoneuron size was studied in homologous single motoneurons innervating the left and right dorsal superficial muscles of the abdomen of the hermit crab, Pagurus pollicarus. Units on the left side discharge at a higher frequency than those on the right. This does not appear to be due to asymmetry in the muscles since these consist of the same number of muscle fibers on each side (although there are a larger number of muscle fibers with mean widths above 125 μ on the left side). Mean diameters of axons above 2 μ were not significantly different on the two sides, nor did the conduction velocities or extracellular amplitudes of homologous motoneurons differ. In addition, pairs of homologous motoneurons of the same size (judged by extracellular amplitudes and conduction velocities) but which innervated different parts of the dorsal superficial muscles, had significantly different frequency ratios. It is concluded that the tonic frequency of bilaterally homologous motoneurons is unrelated to axon size or peripheral field, but may be due instead to central factors.

256 CALCIUM ACTIVATED RECTIFICATION IN SKATE ELECTROPLAQUES. Andrew L. Harris*, John O. Schairer* and Michael V. L. Bennett. Dept. Neuroscience, Albert Einstein College of Medicine, N.Y. 10461.

Electroplaques of Raja exhibit delayed rectification, but no sodium activativation. The conductance increase is evoked by depolarizations of 10-20 mV for 3-5 msec and lasts up to 0.5 sec. Previous work has shown this rectification to be abolished by external barium, and we report here effects of calcium (Ca). The rectification is reversibly abolished by zero Ca in the bath; 0.1mM Ca causes a marked reduction. Intracellular pressure injection of buffered EGTA markedly reduces the rectification, but does not change the resting potential. Injection of Ca solutions causes up to a five-fold drop in resistance, with little or no change in resting potential. Injection of a control buffer solution causes no change in resistance or resting potential. Our present hypothesis is that the rectification depends upon a voltage activated Ca influx that increases the conductance, probably to potassium. This hypothesis is supported by the effects of high Ca solutions. When depolarizing steps are applied, the derivative of the rising phase shows a distinct inflection, suggesting activation of an inward current. Ca dependent rectification has now been found in a number of excitable as well as inexcitable tissues. These results may be particularly relevant to muscle cells, for the electroplaques are myogenic.

257 ASCENDING AND DESCENDING FIBERS IN THE DORSAL COLUMN AT THE MEDULLO-CERVICAL JUNCTION OF A LIZARD AND A SNAKE. Virgil L. Jacobs. Dept. of Anat., Coll. Med., Texas A&M Univ., College Station, Tex., 77801.

The brains and spinal cords of lizards (Lacerta virdis) and snakes (Thamnophis) with medullo-cervical cord lesions were sacrificed after 11-28 days and studied by the Nauta silver method. The ascending and de-descending patterns immediately rostral and caudal to the lesion were compared in the two reptiles. In lizards the ascending degeneration is dorsomedial and occupies more than half of the dorsal funiculus. Below the lesion descending degeneration mainly from the descending tract of V is located in the ventrolateral part of the dorsal funiculus bordering on the medial side of the dorsal horn. In snakes sparse ascending degeneration lies at the dorsomedial periphery and near the dorsal median septum. Below the lesion much of the deeper part of the dorsal column in snakes contains dense degeneration from the descending tract of V. In lizards the increased frontal accumulation of ascending dorsal column fibers displaces the descending tract of V laterally near the dorsal horn. In a limbless reptile like the snake the dorsal column fibers are fewer which allow for the descending tract of V to spread out in the deeper part of the dorsal column. The ascending axons terminate mainly in the dorsal column nuclei while the descending fibers enter the dorsal horn and appear as fine beaded axons in the neuropil.

258 THYLACOLEO, MARSUPIAL LION OR LAMB? AN EXERCISE IN BEHAVIORAL PALEONEUROLOGY. John Irwin Johnson, Biophys.,Psychol., Zool. Depts., Mich. State Univ., E. Lansing, MI 48824 and Leonard B. Radinsky* Dept. Anat., Univ. Chicago, Chicago, IL 60637.

Thylacoleo carnifex is a remarkable extinct marsupial known from Australian Pleistocene fossils. Enormous shearing premolars and enlarged temporal fossae suggest carnivorous habits, unique among large diprotodonts. Gervais in 1869 noted the marked similarities in the shapes of the brains of Thylacoleo and of modern wombats; the sulcal patterns seen in endocasts are almost identical. Electrophysiological mapping studies show a consistent relationship between sulci and sensory projections to cerebral cortex in wombats, and in less similar diprotodonts, wallabies and brushtailed possums. Applying these relations to Thylacoleo, the prominent labial and jugular sulci indicate massive sensory projections from facial, particularly labial and perioral regions; the high jugular sulcus and faint interbrachial sulcus suggest relatively small projections from the limbs; and the absence of a narial sulcus could mean a rhinarial tactile projection smaller than that of wombats, pigs, or coatimundis. Such relatively large labial and perioral projections characterize mammalian herbivores; placental carnivores have relatively greater cortical representation of the limbs. Mapping studies of marsupial carnivores can refine these speculations about the behavior and ecology of Thylacoleo, by revealing whether a small cortical projection from the paws is consistent with a carnivorous life style, perhaps that of a carrion eater like the Tasmanian Devil. If it is not, the hypothesis of Thylacoleo as herbivore is supported by evidence from brain morphology. (Supported by NSF Research Grant GB 43236.)

259 ANTAGONISTIC ACTION OF BARBITURATES AND CALCIUM ON ACTION POTENTIAL REPOLARIZATION IN THE LEECH RETZIUS CELL. Anna L. Kleinhaus and James W. Prichard. Dept. Neurol., Yale Med. Sch., New Haven, CT. 06510.

Phenobarbital, barbital, pentobarbital, secobarbital, methohexital, and thiopental in concentrations of 1-5 mM all reversibly prolonged leech Retzius cell action potential duration to several hundred milliseconds. The prolongation was favored by low Ca and was reversible by elevation of Ca. Barbiturate-prolonged action potentials were unaffected by 50 μM tetrodotoxin or replacement of Cl by propionate but were dependent on external Na. Their amplitudes were increased by steady hyperpolarization and input resistance was reduced during them. They were of shorter duration when elicited at a rapid rate. They were shortened by intracellular iontophoresis of Ca and by use of the ionophore X537A. In contrast to tetraethylammonium chloride- or barium-prolonged action potentials, the barbiturate prolonged events were not shortened by 3 mM manganese chloride, suggesting that current during the plateau was not flowing through the "divalent cation" channel (Kleinhaus, Pflügers Arch. 1976 in press).

A link between an inward Ca current and activation of a K conductance has been described in mammalian and molluscan neurones, although the exact role of this sequence in action potential repolarization varies from cell to cell. The present results are consistent with the hypothesis that in the Retzius cell the barbiturates used blocked a conventional K-dependent repolarization by interfering with a voltage-dependent Ca current necessary for its activation.

260 GOLGI STUDIES OF TWO AVIAN FOVEAS: A QUANTITATIVE ANALYSIS.
Mel Lockhart* (SPON: K. V. Fite). Department of Psychology, Lafayette College, Easton, PA 18042

The concaviclivate fovea of primates appears to mediate high spatial resolution, presumably through connections within a "midget system" (Polyak, 1941). While foveal lesions produce reductions in visual acuity in primates (e.g. Rolls & Cowey, 1970), there is some questions as to whether comparable reductions occur in birds. Pumphrey (1948, 1961) has suggested that the deep, convexiclivate fovea found in birds is used primarily for dynamic acuity, or fixating moving contours, rather than for static spatial resolution.

A Golgi, light microscopic study has focused on the structural relationships in the foveal and peripheral areas of two birds: the bluejay (deep, convexiclivate fovea) and the pigeon (shallow fovea). Although cells in the foveal region were typically smaller than those found peripherally, no "midget system" (midget bipolar-midget ganglion cells) was found. Bipolars with very small (7-10 μ) outer plexiform fields had wider, often multi-laminar, inner plexiform terminations. Ganglion-cell dendritic fields were several times larger than the bipolar inner plexiform layer terminations, even in foveal areas. Furthermore, extensive possibilities for lateral interaction exist in these avian foveas, via receptor-cell basilar processes, horizontal cells and amacrine cells. In general, differences between foveal and peripheral cell dimensions were greater in the bluejay than in the pigeon.

These data suggest that foveal processing in birds may involve several different types of cellular interaction which could code relatively complex stimulus attributes, such as movement and directionality.
(Support by NIH Grant EY 0310 to K. V. Fite.)

261 ELECTRIC ORGAN DISCHARGE AND PREY CAPTURE OF THE STARGAZER (ASTROSCOPUS Y-GRAECUM)
R.F. Martin*, R.B. Leonard and W.D. Willis (SPON: J.S. Kittredge), Marine Biomedical Institute and Dept. of Physiology, Univ. of Texas Medical Branch, Galveston, Texas 77550.

The marine teleost Astroscopus possesses a pair of electric organs developed from extrinsic eye muscles. These electric organs fire characteristic discharges during prey capture. The stargazer lies burrowed in the sand until a fish of prey size enters a discrete region over the stargazer's head. At that time, the stargazer discharges a series of electric potentials, launches up out of the sand to engulf the prey fish, and returns to the sand. The train of discharges continues throughout this activity and for some seconds after its completion. The amplitude of the train portion of the discharge can attain 4.0 volts measured between an electrode implanted in prey fish during capture and an indifferent electrode placed remotely in the aquarium. Film and electrical recordings indicate that the discharge begins several milliseconds prior to capture of the prey. It is presumed that complex sensory input is necessary to enable the stargazer to detect potential prey fish, evaluate the fish as to size and orientation (stargazers preferentially capture prey head-first), and successfully engulf the fish. We found that all stargazers tested fed very effectively in the dark phase of 12-hour day-night cycle. To further evaluate the importance of visual input, the retinas and lenses were removed from the eyes of six stargazers. All of the enucleated fish successfully captured prey and remained healthy. Control and enucleated fish readily strike clean plastic and glass lures, thus excluding chemoreception. The significance of lateral line input and possibility of electroreception are now being investigated. (Supported by NIH Grant NS 11255 and the Moody Foundation of Galveston.)

262 ON THE ORIGIN OF THE AVIAN MESENCEPHALIC NUCLEUS OF THE TRIGEMINAL NERVE. C. H. Narayanan and Y. Narayanan*. Dept. Anat., LSU Sch. Med., New Orleans, La. 70119.

We have reported in a previous study using a modified method of chorioallantoic membrane transplantation that the precursor cells of the avian mesencephalic nucleus of the trigeminal nerve are localized in embryonic midbrain levels. In addition, the results of that study were consistent with the assumption that the neural crest of embryonic midbrain level represents the precursor cells of the mesencephalic nucleus. As a continuation of this study, using quail cells as natural biological markers (Le Douarin, 1970), unilateral transplantation of neural crest cells from donor quail embryos was performed on embryos of duck as hosts. A closely timed stage series of experimental embryos of the duck were sacrificed and fixed in Zenker's solution. The brains were processed for paraffin embedding, serially sectioned at 12μm and stained by Feulgen and Rossenbeck's technique. Histological analyses of sections indicate that all the cells of the mesencephalic nucleus on the operated side on host embryos were derived exclusively from donor quail cells, and were clearly distinguishable from the cells of this nucleus on the unoperated side. The latter were comprised mainly of host cells of the duck. The migration of cells through the leptomeninges as observed in early stages, the clustering and distribution of the cells, and their subsequent maturation in the deeper layers of the optic tectum appeared to be typical of the cells of the mesencephalic nucleus in all cases. These data validate a neural crest origin for the cells of the mesencephalic nucleus which was suggested by chorioallantoic membrane transplantation described previously.

This was supported by USPHS grant #R01 DE04258-01 from the National Institute of Dental Research.

263 CELLULAR ORGANIZATION OF THE OPTIC TECTUM IN ANURAN AMPHIBIANS. Timothy J. Neary. Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109

The distribution of cells in the optic tectum was examined in 12 families of anuran amphibians. The periventricular grey of frogs is generally considered to consist of 3 neuronal laminae (laminae 2, 4, and 6 of Ramon). However, little or no indication of such a cell segregation was seen in two families (Pipidae and Rhinophrynidae). Where a laminar arrangement was present in the periventricular grey, it was not equally distinct in all species studied. In several families (e.g., Centrolenidae, Hylidae) lamina 6 appears to be clearly devided into 2 sublaminae. The number and density of cells in the central grey of the tectum appears to be correlated with the degree of lamination in the periventricular grey. (Supported by NIH Fellowship NS05293)

264 PH-DEPENDENT ACTIONS OF PENTOBARBITAL ON RESTING MEMBRANE PROPERTIES OF LEECH RETZIUS CELLS. James W. Prichard and Anna L. Kleinhaus. Dept. Neurol., Yale Med. Sch., New Haven, CT. 06510.

Pentobarbital, 1-5 mM, caused reversible depolarization and increased input resistance in Retzius cells of leech segmental ganglia. Barbital, phenobarbital, methohexital, and thiopental did not cause such changes; secobarbital did, but less effectively than pentobarbital. Ninety percent replacement of Cl by propionate greatly magnified the input resistance changes. These actions were more pronounced at pH 6.8 than at the usual value of 7.4, and they were absent at 8.8. The characteristic electrophysiological changes were repeatedly reversed by shifting pH back and forth between 6.8 and 8.8 without altering drug concentration. These results are considered to reflect blockade of resting K conductance by pentobarbital and secobarbital. The non-ionized pentobarbital molecule was apparently the active form of the drug, which finding agrees with results obtained by other workers using other excitable membranes. It is unlikely that drug penetration through intervening tissues to the Retzius cell was the process which required the uncharged form of the drug; however, it is possible that access to some site of action inside the Retzius cell depended on movement of the uncharged molecule through the Retzius cell membrane. The same dependency on acid pH was demonstrated for the Ca-reversible block of action potential repolarization caused by pentobarbital, an action exerted by all six barbiturates (Kleinhaus and Prichard, this volume). It is of interest that these two kinds of barbiturate action on the Retzius cell, as well as a phenobarbital-induced increase in resting membrane conductance reported earlier all apparently involve K conductances. Similar phenomena have been reported in other tissues and may be of general neuropharmacological significance.

265 TOPOGRAPHIC ORGANIZATION OF PRIMARY AFFERENTS TO THE SIXTH ABDOMINAL GANGLION OF THE CRAYFISH. R. L. Roth* (SPON: E. E. Sutter). Dept. Biol. Sci., Stanford University, Stanford, CA. 94305.

The axons of primary afferent neurons with connections in the sixth abdominal ganglion of the crayfish, Procambarus clarkii, have been interrupted by partial amputations of the tail fan, partial cauterizations of the tail fan or sixth abdominal segment or transection of individual nerve roots. After postoperative survival periods of 16-96 hrs at 20-22° C., lesioned specimens were fixed in 10% formalin made up in van Harreveld's solution, the abdominal nerve cords embedded in gelatin and 35μm frozen sections processed by a modified Nauta method for degenerating axoplasm.

Central arborizations of primary afferents to the sixth abdominal ganglion are exclusively homolateral, those from different members of the tail fan (or arriving via different nerve roots) occupying sharply delimited and virtually nonoverlapping portions of the ganglionic neuropil. Furthermore, the rostrocaudal and mediolateral relations of the peripheral sensory field are preserved centrally so that the neuropil contains a relatively undistorted map of the exoskeleton.

Whether sensory fibers of different modality (or directional sensitivity in the case of mechanoreceptors) are demonstrably different in their central distribution is not yet known. However, cobalt fills of small populations of afferent fibers show that many axons arborize in an approximately planar fashion parallel to the horizontal dimension of the ganglion. Thus, it may be that the anatomical basis of the modality specificity exhibited by many sensory interneurons lies in a conjoint laminar arrangement of afferent terminals and dendritic arborizations within the depth of the neuropil.

266 SOMATOSENSORY AND VISUAL TOPOGRAPHY IN THE SUPERIOR COLLICULUS OF THE GOLDEN HAMSTER. Sue E. Schneps*, Barbara L. Finlay* (SPON: W.A.Richards)
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Somatosensory and visual receptive fields were recorded electrophysiologically in the superior colliculi of adult hamsters. The topography of the somatosensory projection, which is found principally in the intermediate gray layer, forms a map which is in register with the visual projection in the superficial gray layer. Thus, somatosensory fields for the anterior part of the hamster are found rostrally in the colliculus, where the nasal visual fields are represented. Somatosensory fields along the upper midline of the hamster are found medially in the colliculus, where upper visual fields are represented.

In order for the colliculus to serve as an integrator of visual and somatosensory information for visual orienting behavior, the projections must remain congruent in the presence of both eye and body movements. This problem seems to be solved by differences in the relative specificity of the visual and somatosensory receptive fields. While visual receptive fields are small (less than 10° in diameter for central visual fields), corresponding somatosensory fields subtend at least 30-40° in visual space. Somatosensory receptive fields often encompass a large portion of the hamster body, particularly in the caudalmost fields. Thus, points in the colliculus showing a 20-30° separation of visual receptive fields are correlated with somatosensory receptive fields showing considerable overlap.

267 EFFERENT PROJECTIONS OF THE NUCLEUS ACCUMBENS IN THE TEGU LIZARD. C. M. Sligar* and T. J. Voneida*(SPON: I. Kaiserman-Abramof). Dept. Anat., Sch. Med., CWRU, Cleveland, O. 44106.

The nucleus accumbens (NA) has been variously described as being part of two functionally distinct systems. Thus previous investigators have considered it to be part of the striatal and/or limbic complexes. The present investigation attempts to determine, in the lizard Tupinambis, whether this nucleus is striatal or limbic, or indeed, whether it may contribute to both systems, as appears to be the case in the rat (Domesick et al, 1976) and monkey (Powell and Leman, 1976). A mixture of H^3-Pro and H^3-Leu (15-30 μC) was pressure injected into one NA in each of twelve Tegu lizards. Following a survival period of one week, the animals were perfused, and the brains removed. Efferents from NA were traced to areas within the telencephalon, diencephalon and mesencephalon. Telencephalic areas receiving NA efferents consist of the ventral and ventrolateral striatum, ventral septal area and lateral preoptic area. Within the diencephalon, several thalamic target areas were found, among them the nuclei dorsomedialis and habenularis medialis as well as several other midline thalamic nuclei. Within the hypothalamus, label was widespread and included the lateral and posterior hypothalamic areas, the nuclei dorsalis and periventricularis, and a shell around the nucleus ventromedialis. Mesencephalic projection areas consist of central gray, prerubral field and substantia nigra. All descending projections are via the medial forebrain bundle. The present results, therefore, reinforce the idea that NA influences both striatal and limbic systems by its outflow pattern. (Supported by Grants MH-07051 and EY-04090 from the National Institutes of Health.)

268 AMPULLARY ELECTRORECEPTORS IN STURGEON. J. H. Teeter and M. V. L. Bennett. Albert Einstein College of Medicine, Bronx, N. Y. 10461.

Cutaneous receptors in the shovelnose sturgeon, Scaphirhynchus platorynchus were found to respond to electrical stimuli of less than 1 mV suggesting an electroreceptive function. They look like those on the bill and head of the closely related chondrostean fish, Polyodon spathula (Jørgensen et al., Z. Zellforsch., 130:362, 1972). Weak cathodal stimuli, which make the outside of the skin negative with respect to the inside, accelerate the tonic resting nerve discharge; weak anodal stimuli cause a deceleration. In teleost ampullary organs anodal stimuli directly depolarize the inner (innervated) face of the receptor cell increasing transmitter release and nerve discharge. Cathodal stimuli act oppositely. In ampullae of Lorenzini of elasmobranchs, and apparently in chondrosteans as well, the outer (lumenal) face of the receptor cell is capable of a regenerative Ca response. Thus, cathodal stimuli, which depolarize the outer face, evoke a regenerative response. This depolarizes the inner face and produces an increased transmitter release and nerve discharge. In both elasmobranchs and sturgeon, sufficiently strong stimuli overcome the effects of the response in the outer face and reversed polarity of sensitivity, like that in teleosts, is obtained, i. e. strong anodal stimuli excite and strong cathodal stimuli inhibit nerve discharge. The presence of a regenerative Ca response in sturgeon is supported by the finding that 10 mM $CoCl_2$ in the water bathing the outside of the receptors reversibly blocks nerve discharge. These results are of interest with respect to the evolution of electroreception. In addition, the sturgeon may provide a useful preparation for study of Ca mediated responses.

269 CONNECTIONS OF ANTERIOR DORSAL VENTRICULAR RIDGE IN SNAKES. Philip S. Ulinski. Dept. Anat., Univ. Chicago, Chicago, Ill. 60637.

Anterior dorsal ventricular ridge (ADVR) is a major subcortical, telencephalic structure in reptiles. In snakes, it consists of a cytologically homogeneous population of neurons (Ulinski, J. Morph., 148: 1). These are scattered randomly in the center of ADVR, but form clusters of somata coupled by gap junctions near the periphery. The afferent and efferent connections of ADVR were studied in garter (Thamnophis) and water (Natrix) snakes using Fink-Heimer techniques. Lesions of the midbrain tegmentum produce degenerated fibers in the lateral forebrain bundle (LFB) which terminate ventrally in ADVR. These fibers resemble the Type 1 fibers seen in Golgi preparations. Total thalamic lesions produce degeneration of LFB fibers which terminate in all but the most superficial portion of ADVR. These fibers resemble the Type 3 fibers seen in Golgi preparations. Lesions of the superficial part of ADVR produce both coarse and fine degeneration products within ADVR. The coarse debris resemble the varicosities on type 2 fibers seen in Golgi preparations. These preparations also show that cells in the core of ADVR project to the periphery of the nucleus. Lesions of the core of ADVR produce three fascicles of degenerated fibers. One courses medially from ADVR to terminate in nucleus accumbens. The second enters the LFB to terminate near the intrapeduncular cells. The final fascicle courses laterally to terminate in the nucleus of the accessory olfactory tract and caudally within the posterior dorsal ventricular ridge. These observations indicate that snake ADVR is divided into a core and a periphery which are reciprocally connected. Extrinsic projections from ADVR originate from cells in the core. (Supported by PHS Grant NS 12518).

270 EFFERENT PROJECTIONS OF NUCLEUS SPHERICUS IN THE TEGU LIZARD. T.J. Voneida* and C.M. Sligar* (SPON: H. Gluck). Dept. Anat., Sch. Med., CWRU, Cleveland, O. 44106.

As part of a continuing series of studies of efferent projections from the dorsal ventricular ridge in the Tegu lizard, a H^3-Pro, H^3-Leu mixture (15-30 uC) was pressure injected into the ridge in 12 animals. Injections of nucleus sphericus gave rise to labeled pathways which could be traced rostrally via the lateral olfactory tract to ipsilateral nucleus of the lateral olfactory tract, anterior olfactory nucleus, and granule cell layer of the olfactory bulb. Connections were also found to ipsilateral nucleus accumbens and olfactory tubercle, nucleus interstitialis and marginalis amygdalae. Caudal projections travel by way of stria terminalis to terminate in the ipsilateral peripheral shell of nucleus ventromedialis hypothalamicus. These data will be discussed with reference to recent studies of afferent connections to nucleus sphericus from accessory olfactory bulb in the snake (Halpern, '73) and to caudal amygdala in mammals (Scalia and Winans, '75). The hypothalamic connection from n. sphericus in the lizard will be discussed in relation to earlier behavioral studies of vomeronasal organ and its possible role in feeding and sexual activity. (Supported by Grants MH-07051 and EY-04090 from the National Institutes of Health.)

271 A DETERMINATION OF THE VOLUMES OF THE RAT HIPPOCAMPAL SUBFIELDS. Mark J. West, Gorm Danscher* and Søren Laurberg*. Institute of Anatomy B, Aarhus, Denmark.

The absolute volumes of the subfields of the adult rat hippocampus (e.g. stratum lacunosum moleculare, stratum radiatum, CA1 and CA3 pyramidal cells, mossy fibres, stratum oriens), area dentata (e.g. hilus, granule cell layer, molecular layer) and subiculum have been determined using a laboratory-computer aided plotting device. Series of sections through the hippocampal region were cut either in the horizontal or frontal planes and stained with the Timm sulfide technique for heavy metals. The coordinates of the outlines of the subfields, as they appeared on 30 to 80 sections selected from the series, were input to a mini-computer via a plotter. From these coordinates, the areas of the subfields on the sections were computed and the areas, in turn, used in stereological formulas to compute the respective subfield volumes. The results indicate that the technique can be used for rigorous comparative quantitative studies of the rat hippocampal region since most of the subfield volumes had standard errors which were less than 4% of the mean volumes (N=4). In addition to the volumetric data, descriptions of the computerized plotter and stereological formulas and an evaluation of the sensitivity of the technique as applied to the rat hippocampus will be presented.

Development and Aging

272 DEVELOPING PREFRONTAL CORTEX: EFFECTS OF COOLING ON BEHAVIOR AND ON SUBCORTICAL NEURONAL ACTIVITY IN RHESUS MONKEYS. G. E. Alexander and P. S. Goldman. NIH, Bethesda, MD. 20014.

In adult rhesus monkeys, bilateral lesions of the dorsolateral prefrontal cortex (DLC), the head of the caudate nucleus (HCN), or the dorsomedial nucleus of the thalamus (DM) all lead to reliable deficits in performance on delayed-response tasks (DR). By contrast, in monkeys operated as infants, DR impairments can be obtained only following damage to the subcortical structures, while DR performance is unaffected by dorsolateral cortical removal. These and related observations have led to the hypothesis that the dorsolateral cortex does not assume a significant role in mediating DR performance in yearlings, and that either DM or HCN, or both, might have a preeminent part in subserving such behaviors prior to the maturation of the cortex.

In order to examine this question, we have employed reversible cryogenic depression of DLC, in conjunction with subcortical single unit recording, in trained animals of various known ages. Following training on a DR task with a 12 second delay, the animals were implanted with chronic recording pedestals oriented for subsequent single unit analysis of DM and HCN. Stainless steel cooling chambers and thermistors for monitoring subdural temperatures were implanted over each prefrontal convexity. Bilateral DLC cooling was standardized so that in each animal the cortical surface temperature during cooling was 20°C directly beneath each chamber. Isotherms were compiled from depth temperature readings to insure that only the DLC and underlying white matter were significantly affected by cooling. In particular, a temperature of 37°C was recorded in both DM and HCN during DLC cooling to 20°C.

HCN units behave similarly in all age groups in relation to events of the DR task. The typical HCN unit increases its firing rate during the cue period and the response period, but not during the delay between the cue and response. In contrast, increased firing during the delay period is seen in DM units, and such increases are evident at all ages. Furthermore, in all age groups DLC cooling changes unit firing in DM and HCN, and these changes are entirely reversible.

DLC cooling in 13-15 month old animals does not influence DR performance. The 23-25 month old animals show small but reliable cooling-related decrements of approximately 5% in their performance levels. Finally, in agreement with earlier studies in young adults, by 33-35 months of age the animals exhibit mean DR performance decrements of approximately 25% in association with prefrontal cooling. All such deficits are completely reversed by rewarming the DLC to 37°C.

The finding that DLC cooling does not produce disruption of DR behavior in monkeys until they are young adults provides further evidence that the dorsolateral prefrontal cortex only gradually develops its cognitive functions over the first two years of life. Moreover, the delay-related increases in unit firing observed in DM in infants as well as adults is consistent with the possibility that this nucleus is involved in the mediation of DR behavior prior to maturation of the DLC. However, the precise role of the HCN in such behavior remains to be elucidated. Finally, the results indicate that the DLC exerts an influence on subcortical neuronal activity even before its role in mediating behavioral processes is fully developed.

273 MICROSPECTROFLUOROMETRIC, MICROFLUOROMETRIC AND HISTOCHEMICAL STUDIES OF NEUROMELANIN AND LIPOFUSCIN IN THE BRAIN OF HUMANS AND THE DOG.

Herbert Barden, Francisco Aviles* and Stewart Levy*. Dept. Neurotoxicol., N.Y.S. Psychiatric Inst., New York, N.Y. 10032.

Oxidative bleaching of the naturally dark intraneuronal mass of neuromelanin granules converts this pigment into a colorless and fluorescent residuum suggestive of lipofuscin. This study was undertaken to further elaborate the nature of this residuum.

Untreated, extracted or bleached sections, and both extracted and bleached sections of human brain containing the age pigments neuromelanin in the substantia nigra, and lipofuscin in the inferior olive, were compared using microfluorometry and microspectrofluorometry for wavelengths between 450 and 700nm. Tinctorial histochemistry on similarly prepared sections included the periodic acid-Schiff and direct Schiff reactions as well as the cupric ion- and ferrous ion-uptake reactions. These staining reactions were undertaken following prior bromination, acetylation, saponification and carbohydrate digestion procedures. Neuromelanin in hypothalamus of dog brain was also stained by some Schiff procedures.

Microspectrofluorometry revealed a fluorescence level always higher at the shorter wavelengths. The curve for bleached neuromelanin demonstrated a fluorescence level midway between that of lipofuscin, which in all instances gave the greater amount of fluorescence, whether it was treated or not, and that of the neuropil adjacent to neuromelanin, which gave the least amount of fluorescence. Microspectrofluorometry of the extracted and bleached residuum of neuromelanin and similarly treated lipofuscin demonstrated little change in the latter instance whereas the fluorescence of the neuromelanin residuum remained evident though seemingly obscured by an enhanced tissue fluorescence including that of the perikaryal cytoplasm and adjacent neuropil. Microfluorometric quantitation of the fluorescence of treated and untreated neuromelanin, lipofuscin and neuropil was confirmatory and gave a ratio of approximately 4:2:1 for the fluorescence of lipofuscin, the residuum of neuromelanin and neuropil. Each Schiff reaction was of moderate intensity in the bleached residuum of neuromelanin and of weaker intensity in the extracted and bleached residuum. Lipofuscin did not give a direct Schiff reaction under any of the conditions utilized in this study. Prior acetylation prevented any of the four tinctorial reactions in tissue prepared by any of the methods while saponification of the acetylated sections reversed this inhibition in each instance. Tinctorial reactions run in sections of dog were similar to those of human.

While these results demonstrate similarities in the fluorescence characteristics of the bleached residuum of neuromelanin, the extracted and bleached residuum of neuromelanin, and lipofuscin, the presence of a direct Schiff reaction in the residuum indicates that this component of the bleached neuromelanin granule is a lipofuscin having an atypical composition.

274 THE ROLE OF NGF IN THE DEVELOPMENT OF RAT SYMPATHETIC NEURONS IN VITRO. Linda L.Y. Chun* and Paul H. Patterson* (SPON: Dennis M.D. Landis). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

Pure neuronal cultures offer a useful system for study of the role of nerve growth factor (NGF) because (i) effects are attributable to a direct action of NGF on the neurons themselves, (ii) biochemical analyses are not contaminated by non-neuronal cell constituents, and (iii) non-neuronal influences which may complement or compete with NGF are absent. The development of rat sympathetic neurons in cultures virtually free of other cell types was studied as a function of 7S NGF concentration. Both neuronal survival and catecholamine production were greater with increased levels of NGF. Assayed at 3 weeks, neuronal survival increased exponentially from 0 to 9000 somas/dish as the NGF concentration was varied from 0 to 5 µg/ml. The ability to synthesize and accumulate ^{3}H-catecholamines (CA) from ^{3}H-tyrosine was 6-fold higher per cell in neurons grown in 5 µg/ml than those grown in 0.01 µg/ml. However, the rate of protein synthesis from tyrosine did not increase per cell over the same NGF concentration range. If the rate of protein synthesis reflects overall neuronal growth, then these results are consistent with the idea that NGF can cause dose-dependent increases in differentiation as well as survival.

In the presence of cells from neonatal rat heart the NGF requirement for neuronal survival was partially spared; that is, some neurons survived even in the absence of exogenous NGF. Furthermore, the level of cell survival was dependent on the density of heart cells present, supporting the hypothesis that non-neuronal cells can produce NGF (Young, et al., Sci. 187, 361 and Burnham, et al., PNAS 69, 3556). Acetylcholine (ACh) synthesis from choline, which is induced in the neurons by the heart cells or medium conditioned by them (Patterson, et al., CSHSQB 40 (1976)), and CA production were both increased by NGF with similar dose-dependencies. In addition, the neurons require exogenous NGF for survival, at least until day 20, both under conditions where the neuronal population (i) produces CA almost exclusively (no heart cells present) or (ii) produces predominantly ACh (grown in high concentrations of medium conditioned by heart cells). In both populations two-thirds of the cells die if at day 20 the NGF is omitted from the media for 10 days. Furthermore, those cells still remaining at day 30 show decreased CA and ACh production per neuron. Thus with respect to NGF requirements, these cholinergic cells are more like sympathetic than parasympathetic neurons. (Supp. by NIH and the American and Massachusetts Heart Associations)

275 A COMPARISON OF "SPONTANEOUS" AND INDUCED CELL DEATH IN THE LUMBAR SPINAL CORD OF THE CHICK EMBRYO: AN ELECTRON MICROSCOPIC STUDY. I-Wu Chu-Wang and Ronald W. Oppenheim, Dept. of Mental Health, Raleigh, N. C. 27611

A few spontaneously degenerating motoneurons can be detected as early as 4 days in the chick embryo. All the motoneurons at this stage are immature with a small amount of cytoplasm, abundant free ribosomes and a few endoplasmic reticulum. The degeneration process must be very rapid at this early stage since so far we have only observed late lytic debris of dead cells in the extracellular space of the lateral motor column (LMC). A more massive degeneration occurs between day 6 and day 9. During these 3 days, 40% of the neuron population disappears. The dying cells undergo shrinkage and condensation. Morphologically, two types of initial degeneration can be recognized. Type 1: The majority of ribosomes are dissociated and free in the cytoplasm. Small pieces of cisternae of rough endoplasmic reticulum are still recognizable. Most of the mitochondria are vacuolized. The nucleus contains condensed chromotin. Type 2: The most characteristic feature is the striking dilatation of endoplasmic reticulum, nuclear envelope and Golgi apparatus. Ribosomes still form distinctly rosette-like polysomes. Very few mitochondria show degeneration signs. In the advanced phase, the nuclear envelope breaks down and the nuclear content is mixed with lytic cytoplasm. The dilated membrane system occurring in type 2 later breaks down into vesicles some of which still have ribosomes attached. The dying cells finally autolyze into highly osmophilic and compact debris which are then phagocytozed by radial ependymal processes, multipotential glial cells and mononuclear leukocytes.

In the case of induced cell death by removal of the limb bud on day 2.5 we found that both types described above occur in the LMC. The only difference is that limb bud removal increases the speed and the number of cells undergoing degeneration. By day 9, about 90% of the cell population of the LMC has degenerated.

By quantitative comparison of cell number in the LMC with axon number in ventral roots of normal chick embryos from day 4 to post-hatch, we found that there is an approximate one to one ratio between the numbers of motoneuron cell bodies and axons before, during and following the period of maximal spontaneous cell death. In order to prove that motoneurons already send axons to the limb musculature prior to their spontaneous death, we injected horseradish peroxidase (HRP) into the limb on days 5-7 of incubation and sacrificed 24 hrs later. Cells were found in various stages of degeneration which contain HRP reaction product. It is suggested that these cells which have undergone spontaneous cell death in the 24 hr period following injection had already sent axons to the limb musculature.

276 AGE RELATED LATENCY CHANGES IN EEG VISUAL EVOKED POTENTIAL COMPONENTS WITHIN AND ACROSS SUBJECTS. Sarah L. Friedman* N.I.M.H. Bethesda, MD. 20014. Ann B. Barnet, Ira P. Weiss and Elizabeth S. Ohlrich*. Children's Hospital National Medical Center, Washington, D.C. 20009.

Repeated recordings from fifteen children between the time they were two weeks and three years of age revealed ten relatively reliable EEG visual evoked potential (VEP) components across age and across subjects. While short-latency components remained relatively constant across age, long-latency components progressively approached latency values generally reported for adult VEPs.

Ten to seventeen VEPs were obtained from each of the fifteen unsedated sleeping subjects. The stimulus set consisted of 100 intense white light flashes presented 2.5 seconds apart. EEG from O_z to joined mastoids for one post-stimulus second was averaged over the stimulus set to yield each VEP.

VEPs of each subject were rank-ordered by age and the components were marked with an attempt to give apparently homologous components within a subject's records the same label. The number of components in each individual's record increased with age. For example, the mean number of components in the first 120 days was 6.4 and in the following 120 days 8.4. The number of components that characterized a subject's most complex VEP was between nine and twelve. When only components which occurred in more than half a subject's record were considered, a minimum of seven and a maximum of ten components were identified. The mean latencies of the components of the different subjects fell within a limited range such that there was no overlap among the ranges of the various positive or negative components. The most frequently occurring component was a negative one with mean latency of at least 250 and at most 390 msec. The latencies of the early components corresponded well with those reported for adults. The latencies of the later components approached adult values by the time the subjects were two to three years of age.

The change in VEP latency over age was indexed by correlating each component for each subject with age. The latency of early components (appearing between 20 and 100 msec. post-stimulus) did not change systematically over the three years period. The latency of later components (appearing between 115 and 750 msec. post-stimulus) decreased significantly over the same period. There were differences among subjects in the number of components which showed a significant age related latency change. Thirty-three, 17, and 28 percent of the subjects showed a significant decrease in latency for the three early components. Between 62 and 88 percent showed such a decrease for their late VEP components.

Neurophysiological and histological research suggest that early VEP components are the product of brain activity in the specific sensory cortex while later components represent activity in later to develop brain areas or in areas that are late to connect with area 17. If this is the case, then the VEP findings of the present study reflect the differential development of sensory and cognitive function in the first three years of life.

277 LOSS OF SYNAPSES AND DECREASED AXONAL TRANSPORT OF GLYCOPROTEINS IN THE SEPTO-DENTATE PATHWAY OF THE SENESCENT RAT BRAIN. Yuri Geinisman* and William Bondareff. Dept. Anat., Sch. Med., Northwestern University, Chicago, IL. 60611.

A correlative study of the ultrastructure of synaptic contacts and of axonal transport of glycoproteins destined for these synapses was undertaken to test the hypothesis that the phenomenon of age-related decrease in numbers of synapses, described by us earlier (Mech. Age. Devel. 5:11, 1976) for the middle third of the dentate gyrus molecular layer in the Fischer 344 rat, is associated with a reduction in axonal transport. Young adult (3 month old) and senescent (25 month old) Fischer 344 rats were compared.

For studies of synaptic ultrastructure, the cranial portion of the right dentate gyrus was dissected from perfused brains of 5 young adult and 5 senescent rats. The dorsal blade of the dentate gyrus was sectioned coronally and electron micrographs (120 per rat) were obtained at random from the supragranular zone of the dentate gyrus molecular layer where axons from the medial nucleus of the septum are known to form synaptic contacts on dendrites of dentate neurons. The ultrastructure of the axo-dendritic synapses was found to be identical for animals of both age groups, but the number of synapses decreased significantly in senescent rats relative to young adults.

For studies of axonal transport, ^{3}H-fucose was injected stereotaxically into the right medial nucleus of the septum in 35 young adult and 25 senescent rats. At various time intervals (10-60 min) after injection, the tissue of the medial septal nucleus and of the cranial portion of the right dentate gyrus was dissected free and homogenized. Protein was precipitated with cold TCA-PTA, and both the protein fraction and the supernatant counted in a liquid scintillation counter. Although there was no difference in the percentage of ^{3}H-fucose incorporation into glycoproteins of the medial septal nucleus between young adult and senescent rats, ^{3}H-fucose-labelled glycoproteins appeared to be transported axonally from the medial nucleus of the septum to the dentate gyrus at a significantly lower rate and amount in senescent rats compared to young adults.

These results, taken together, suggest that the loss of synapses characteristic of senescent rat brain may be due, at least partially, to a decline in axonal transport of glycoproteins required for remodelling of synaptic structures.

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278 DIFFERENCES IN DEVELOPMENT OF ADULT RELATIVE EYE POSITION IN XENOPUS AND RANA. Paul Grobstein and Christopher Comer*. Dept. Pharmacol. and Physiol. Sci., Univ. of Chicago, Chicago, Ill. 60637.

In adults of both Rana pipiens and Xenopus laevis the visual fields of the two eyes overlap substantially. In tectal regions corresponding to areas within this binocular field, activity can be evoked from the same visual field position through either the contralateral or the ipsilateral eye. In both animals the ipsilateral visuo-tectal (indirect) projection first is detectable during metamorphosis when the eyes migrate from their larval position with little or no binocular field to the more frontal and dorsal position characteristic of the adult. Experimental rotation of one eye in late larval Xenopus apparently results in a modification of the ipsilateral visuo-tectal projection, assayed post-metamorphically, such as to realign it with the rotated contralateral visuo-tectal projection (Gaze, R.M. et al., Proc. Roy. Soc. Lond. B 175: 107, 1970). Additional experiments support the original suggestion that visual experience is involved in matching contralateral and ipsilateral projections in Xenopus (Keating, M.J. British Med. Bull. 30: 145, 1974). In Rana pipiens, however, modification of the ipsilateral projection consequent on contralateral eye rotation has been reported not to occur (Jacobson, M. Proc. Natl. Acad. Sci. USA 68: 528, 1971).

We have investigated the time courses over which the development of adult relative eye position occurs in Rana and Xenopus by using an optical technique (slightly modified from Fite, K.V. Behav. Biol. 9: 707, 1973) to establish the boundaries of the visual fields of the two eyes with reference to body axes for both organisms at ages from immediately post-metamorphosis to adulthood. Our basic finding in Xenopus is to confirm that a large amount of eye migration occurs subsequent to the end of metamorphosis (Keating, 1974). The migration is mostly dorsal, decreases the divergence of the optic axes by in excess of 30° and is only half-completed in animals two months post-metamorphic. By contrast, in Rana we have found that relative eye position at the end of metamorphosis is very close to that of the adult. There seems to be a slight subsequent anterior migration (less than 5° for each eye) but it is just at the limit of detectability with our technique.

These findings indicate that Rana and Xenopus may have quite different developmental problems to solve with respect to binocularity and hence provide possible explanations for the discrepant experimental results in the two organisms. Keating (1974) has suggested that visual experience is used to maintain proper binocular interaction during continued eye migration in Xenopus. One possible explanation for the discrepant experimental results, in light of the present findings, is that Rana does not have to cope with significant eye migration and may therefore lack a mechanism for making use of visual experience to match contralateral and ipsilateral projections. A second possibility is that both Rana and Xenopus have an experience-dependent mechanism for assuring proper binocular fusion but that they differ in the amount of variation in relative eye position for which they can compensate, the differences being in accord with the normal variation present during the developmental process. The experimental demonstration of an altered ipsilateral projection has depended on fairly large contralateral eye rotations which, given the present results, may be well outside the normal range of variation in Rana.

279 DEVELOPMENT OF NEOCORTICAL CIRCUITRY IN THE RAT: AN ULTRASTRUCTURAL AND GOLGI ANALYSIS. Donald A. Kristt and Mark E. Molliver, Departments of Anatomy, Neurology and Division of Neuropathology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

The purpose of this correlative study is to further describe developing neuronal circuitry in somesthetic cortex of the neonatal rat. Using a quantitative electron microscopic technique, we have previously shown that immature cortex is characterized by tangential strata of high and low synaptic density. At birth (P-0) two strata of high synaptic density are present: one in the molecular layer and one in the cell sparse region between the cortical plate and immature white matter, viz. subplate layer (SPL). By the end of the first week (P-6) a third synapse-rich stratum appears in the lower, one third of the cortical plate. Based on this stratiform distribution of synapses we hypothesized that morphology of synapses might differ between strata. A preliminary ultrastructural analysis revealed that there were morphological (Br. Res. 108, 1976) and cytochemical (Neurosci. Letters 1 (1975) differences between synapses in different strata. Although, the post-synaptic elements were identified as either a proximal or distal dendrite or soma, there was little information regarding the type, characteristics or maturity of post-synaptic neurons. We have therefore utilized rapid Golgi methods to study neurons in mid-lateral (somesthetic) cortex of neonatal rat.

In the P-0 rat, the molecular layer is a region characterized by tangentially and obliquely running processes. These are: (1) axons and (2) terminations of apical dendrites of somata lying in the cortical plate and SPL. Our ultrastructural observations reveal that many of the post-synaptic elements in the molecular layer are apical dendrite branches. In contrast, in the synaptic stratum in the SPL, synapses on somata, proximal dendrites and small diameter profiles are common. The Golgi analysis reveals that the most differentiated neuronal elements in P-0 cortex are located within the SPL stratum, viz. (1) pyramidal somata (future layer V), (2) their proximal dendrites and (3) apical dendrites of plexiform layer cells. Also, several spines were observed on the proximal dendrites of these layer V somata. Based on our ultrastructural data, it is likely that many of these 'relatively mature' elements are post-synaptic in SPL.

In the P-6 rat, the molecular layer was similar to that in the P-0 rat. In the cortical plate and SPL, basal dendrites of neurons were more mature than at P-0 in that there were: (1) greater numbers of dendrites/cell, (2) greater extent of the dendritic field and (3) branching was more common and complex. In a given neuron (at P-6), some basal dendrites were more mature than others in terms of length, branching complexity and spine density. Typically, the most mature basal dendrites of a neuron were directed towards or ran within one of the two deep synaptic strata. For example: (1) Neurons with somata just above the synaptic stratum in the cortical plate have two prominent basal dendrites that run obliquely downward; these dendrites are moderately long and sparsely covered with spines. (2) Somata within the cortical plate stratum give rise to numerous short, tangential dendrites. (3) Just beneath the cortical plate, star pyramids radiate relatively long dendrites upwards (adpial) into the cortical plate stratum. The deepest synaptic stratum in the SPL contains the largest pyramids in P-6 cortex. Their lateral running dendrites remain within this stratum and are the most highly branched basal dendrites of these cells. They are covered with more spines than any of the other dendrites of these cells (although many fewer spines than seen in the adult). Hence, in many neurons in P-6 cortex, there is a spectrum of dendritic maturity, and a cell's most mature basal dendrites approach, traverse or run within the nearest synaptic stratum.

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280 SYNAPSES FORMED BETWEEN DISSOCIATED SYMPATHETIC NEURONS: THE INFLUENCE OF CONDITIONED MEDIUM. Story C. Landis, P.R. MacLeish*, D.D. Potter*, E.J. Furshpan* and Paul H. Patterson*. Dept. of Neurobiol., Harvard Medical School, Boston, MA 02115

Neurons dissociated from the superior cervical ganglia of newborn rats were grown in the absence of other cell types. Cultures that synthesized (1) predominantly norepinephrine (NE), (2) predominantly acetylcholine (ACh), or (3) significant amounts of both neurotransmitters were obtained by adding varying amounts of medium conditioned by rat heart cells (Patterson, Reichardt and Chun, CSHSQB 40, 1976). After 3 weeks in vitro, the incidence of cholinergic synapse formation was estimated electrophysiologically, and the synapses and varicosities were examined with the electron microscope in sister cultures fixed with permanganate to localize NE stores as small granular vesicles (SGV).

Cultures grown in unconditioned medium (0% CM) that synthesized almost exclusively NE contained few or no physiologically identified cholinergic neurons or "drivers" (see O'Lague et al., CSHSQB 40, 1976); only 1% of the synapses and varicosities sampled had no SGV. In contrast, in cultures grown in high concentrations of CM and that synthesized predominantly ACh, a large proportion of the neurons were identified as cholinergic drivers; 78% of the terminals contained no SGV but only clear vesicles. When intermediate levels of conditioned medium were used and both NE and ACh were synthesized in significant amounts, some but not as many cholinergic drivers and terminals with no SGV (1-46%) were present. Thus as the concentration of CM used and the amount of ACh synthesized by a culture increased, so did the number of physiologically identified cholinergic driver neurons. Whatever cues are provided by CM, they not only give rise to the synthesis and accumulation of substantial amounts of ACh but can induce the formation of large numbers of functional cholinergic synapses. As the concentration of CM increased, a concomitant increase was observed in the proportion of synaptic endings and varicosities that contained no SGV.

The terminals that contained SGV had varying proportions of small granular and clear vesicles. Incubation with 10^{-5}M 5-hydroxydopamine (5OHDA), a NE congener, prior to permanganate fixation increased the proportion of SGV in these terminals. In addition, incubation decreased the proportion of terminals that contained no SGV. This suggests that many terminals which contained no SGV when only endogenous NE was localized (and which would be characterized as cholinergic in tissues such as the iris) can take up and store at least small amounts of catecholamines. Studies are currently in progress to determine whether the terminals of identified cholinergic driver neurons contain SGV either before or after application of 5OHDA. (Supported by NIH research grants NS03273, NS11576 and NS02253 and postdoctoral fellowship NS04093)

281 ELECTRON-DENSE INTERCELLULAR MATERIAL IN WIDE-GAP APPOSITIONS BETWEEN NEURONS IN CHICK-EMBRYO TELENCEPHALIC AGGREGATES. Luis M. H. Larramendi* and Beatrice Garber. Dept. Anat. and Biol., Univ. of Chicago, Chicago, Ill. 60637.

Aggregates prepared in vitro from dissociated cells of chick embryonic telencephalon have been shown with Golgi techniques to consist of various types of neurons and glial cells (Palacios, Garber and Larramendi, 1973). Electronmicroscopic studies of these aggregates and others from isolated cortex of 10-day old embryos, cultured for 1-8 days, have shown - in addition to synapses, junctions and regular cell contacts- a special type of apposition referred to here as wide-gap apposition (WGA). These appositions are separated by an intercellular space 400 to 500 A° wide containing a moderately electron-dense material which tends to form an intracleft line. WGAs have been observed between neuronal somata, somata and dendrites, and dendrites. So far, they have not been observed between axons or boutons and other structures, between well characterized glial cells, or between glia and neurons. The extent of these WGAs varies with the size of the apposed surfaces, ranging from one to several microns. WGAs represent only a small fraction of all cell contacts observed in aggregates. Often it is observed that one single structure forms WGAs with several neighboring elements (clustering effect) which in turn may form only regular cell contacts with other structures. The intercellular material observed in WGAs has never been seen on free unapposed cell surfaces. WGAs are well formed within 24 hours of the aggregation and are prominent after 6 days in culture when cell differentiation reaches its peak. Since this unique and intriguing special type of cell apposition has not yet been observed in developing CNS in situ or reported in monolayers or explants in vitro but its presence is correlated with successful histogenesis and cell differentiation in aggregates, it does appear that the intercellular material in WGAs may play an important role in the aggregation and/or stabilization of neuronal assemblies organized in vitro from dissociated embryonic cells. The nature of the material in these WGAs, the factors responsible for the formation of these appositions and the clustering effect mediated by WGAs are under investigation.

282 HISTOGENESIS OF SEPTAL NUCLEI OF THE RAT BRAIN WITH SPECIAL CONSIDERATION OF THE NUCLEUS ACCUMBENS. S. N. Lawson*, M. K. May* and T. J. Biscoe* (SPON: W. W. Kaelber). Dept. of Physiol., University of Bristol, The Medical School, Bristol, Eng. and Dept. of Anat., University of Iowa Medical School, Iowa City, IA 52242.

Tritiated thymidine autoradiography shows that the onset of neuroblast formation occurs at about 12 days gestation in all septal nuclei studied. Peak activity was found at 13.5 days in those nuclei which lie closest to the midline (medial and triangular nuclei) and at 15.5 days in those nuclei positioned more laterally (lateral and accumbens nuclei). This suggests a medio-lateral gradient of neuroblast formation within the septal region.

Our data also suggests an additional wave of activity in the accumbens nucleus. Das and Altman (Brain Research 21:122, 1970) have reported a peak of activity reached at birth (22 days gestation) which may correspond to a continuation upwards of our second peak. The possibility that if two waves of activity exist they may involve different cell populations as seen in the adult was investigated by measuring nuclear areas of the cells labelled at the maximal points of final cell division observed in the present study, that is on 15.5 and 19.5 days gestation.

Outlines of nuclei were drawn using a drawing tube apparatus and the areas were measured by using an electromagnetic cursor (D.MAC) to follow nuclear circumferences. The cursor continuously samples X and Y coordinates and was coupled to a PDP8 computer the output of which gave the area enclosed in the circumference. The areas were subsequently sorted into bins. Using cumulative probability plots of these frequency distribution histograms on probability paper as described by Harding (J. Marine Biol. Ass. 28:141, 1949) it is possible to estimate the number of normal distributions and the proportion comprising each. A χ^2 test shows the goodness of fit between the expected distribution estimated as above, and the observed distribution. The populations described below gave values of $P>.05$, i.e. not significantly different.

Two distinct populations of cells were found at 15.5 days; 91% had nuclei with a mean area of $86\mu m^2$ and 9% had larger nuclear areas with a mean of $147\mu m^2$. At 19.5 days, however, the labelled cells are part of a single population as regards nuclear area; the mean area is $84\mu m^2$. It therefore appears that the majority of the labelled cells at both ages were of the same cell type, that is with smaller nuclei. At the earlier age of 15.5 days, however, an additional population of neurons with larger nuclei was undergoing the stage of final cell division and these had ceased division by day 19. The 15.5 day peak may, therefore, represent the peak of formation of the larger cells superimposed on the increasing rate of formation of the smaller cells.

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283 EFFECT OF OLFACTORY LESIONS ON BEHAVIORAL DEVELOPMENT IN HAMSTER PUPS. Christiana M. Leonard. Dept. Anat., Mt. Sinai Sch. Med., CUNY, N.Y. 10029

Hamster pups are virtually poikilothermic during the first week of life. Their core temperature is maintained at 38°C through continual close contact with their mother. When removed from the nest their temperature drops rapidly. If exposed to a thermal gradient they orient quickly and reliably to the warm side and locomote rapidly, stopping when their core temperature starts to rise (usually at air temperatures between 33° and 37°C). Although 8 to 10 day old pups also lose heat when removed from the nest they do not show a reliable thermal preference. Rather they wander around slowly, swinging their noses from side to side, apparently oblivious to the thermal qualities of the environment. Olfactory bulbectomy prevents this loss of thermal responsiveness.

Litters of 3 and 7 day old pups were immobilized with ice and received bilateral bulbectomies (BOB, n=10) or unilateral bulbectomies (UOB, n=10). Control litters (n=11) were immersed in ice for an equivalent period or left undisturbed (n=6). Pups were tested daily on a thermal gradient (22° to 36°) for one minute.

Table I

Percent time on hot side (median of group)

Age of test (days)	3 - 5	6 - 7	8 - 10	11 - 13
Controls	86	80	25	7
BOB			82*	60*
UOB			46*	27*

*Distribution significantly different from other two groups at .05 level (Kolgomorov-Smirnov test)

In a second experiment UOB pups showed an even higher thermal preference when the test was extended for two minutes. In this experiment belly and rectal temperatures were monitored during thermal testing. Bulbectomized pups allowed their temperatures to rise more rapidly and to higher levels than controls.

One can interpret these results in terms of sensory competition. The young hamster's behavior is dominated by thermal stimuli. As his olfactory system matures odors come to compete with thermal stimuli for control of behavior. At this stage even subtotal lesions (e.g., unilateral bulbectomy) have an effect in unbalancing the system and pushing behavior back to a more primitive mode of thermal dependence. There is independent evidence that seven days is a critical time for olfactory maturation. This is the age at which olfactory preference is first demonstrated for maternal shavings (Devor and Murphy, 1973) and when fiber degeneration is first seen in the medial structures innervated by the lateral olfactory tract. The maturation of these olfactory projections may be responsible for the inhibition of thermal behavior at 8 days of age. This possibility has been investigated by Small (see abstract, this volume) using a recovery of function paradigm. (Supported by grant NS12458).

284 A COMPARATIVE AUTORADIOGRAPHIC STUDY OF EARLY NEURON ORIGIN IN THE MOUSE AND CHICK. J. A. McConnell and J. W. Sechrist (SPON: R. P. Gruener). Dept. Anat., Coll. Med., Univ. of Ariz., Tucson, AZ. 85724.

In the 1930's Windle and colleagues suggested that the early behavioral differences between the chick and the mammal are due to variations in the sequential appearance of fundamental neuron types. Integrator neurons were said to be precocious in chick embryos whereas primary motor neurons differentiate first in the mammal. The specific objectives of this study were to re-evaluate this concept by determining when the first neurons originate in the mouse embryo as was previously reported for the chick (Sechrist, Anat. Rec. 181: 474, 1975) and by localizing these neurons in the more mature chick and mouse.

Data was obtained from BALB/c mouse embryos by cumulative labeling with ^{3}H-thymidine. Pregnant females were given three intrauterine injections (3-5μCi per implantation site) of the isotope during a 10 hour period on embryonic day 8, 9 or 10. Observations based on both paraffin and epoxy embedded sections of the embryonic neuraxis indicated a few postmitotic neurons are present at least by the 8 somite stage (E8½) prior to neural tube closure. Thus some neuronal precursors are undergoing final DNA synthesis early on embryonic day 8 when the first somites are forming. A 16 somite embryo cumulatively labeled during the 10 hours prior to fixation contained unlabeled cells (young neurons) in the brainstem and rostral spinal cord. These cells were located in both alar and basal plates with no more than 1-3 per section. Older embryos treated similarly provided confirmation for several distinct differences between the chick and the mouse. Not only does neuron origin begin later in the mouse (3-6 somite stage vs. late intermediate streak stage) but the manner in which cells exit the proliferative zone is clearly different. Postmitotic basal plate cells predominate in the mouse from the beginning although a very few alar plate cells appear at about the same time. Observations of 27 somite embryos from both species cumulatively labeled 10 hours earlier indicate cranial nerve motor nuclei are further developed in the mouse. Even more obvious at cervical levels are the presence of ventral root fibers, a prominent unlabeled motor column, a markedly narrowed basal plate ventricular zone, and many postmitotic cells in dorsal root ganglia. At the same stage and levels, the chick has only a thin peripheral zone of unlabeled cells and no ventral roots.

Localization and identification of these early neurons were made in postnatal day 30 mice which were pulse labeled (1 intrauterine injection) at regular intervals on E8, E9 or E10 and in E18 chicks cumulatively labeled (2 injections 2½ hours apart) at similar intervals between 20 and 72 hours. Extensive comparisons have been made using one animal of each species treated about the 10 somite stage (E8½ mouse; 32hour chick) Autoradiographs of the neuraxis indicated that in the brainstem of both the mouse and chick widely scattered small and medium sized reticular neurons (3-4 per section) originate first. Within 2-4 hours in the mouse, primary motor neurons of the visceral efferent column begin to originate; equivalent motor cells in the chick lag by at least 12 hours. At cervical levels of the mouse spinal cord heavily labeled small and medium sized neurons are found on the periphery of the ventral horn. The chick shows the same pattern, with the addition of similar cells occurring within the ventral horn and the intermediate zone and at the lateral border of the dorsal horn. In both species morphological characteristics suggest that these are interneurons (perhaps commisural or propriospinal). The large primary motor neurons are more lightly labeled in the chick than in the mouse, evidence that the chick cells have undergone more divisions. Our impression is that the chronology of origin of motor and integrator nerve cells is not reversed for the two species, but that precocious origin of mouse motor elements is superimposed on a pattern of interneuron origin similar, if not identical, to that of the chick.

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285 CHRONIC DEFICITS IN THE EMBRYONIC MOTILITY OF THE CHICK AFTER SHORT-TERM (24 HR) IMMOBILIZATION WITH CURARE. Ronald W. Oppenheim, Marjory J. Gray* and Randy Pittman*. Neuroembryology Lab., Dept. of Mental Health, Raleigh, N. C. 27611.

On day 10 of incubation chick embryos were immobilized by either a single injection or a continuous infusion of a total of 4-mg of d-tubocuraine. Embryos treated in this way remained totally immobilized for 20-30 hrs as determined by periodic direct observations. Beginning 24 hrs after the return of neuromuscular activity (i.e. on day 12), the spontaneous motility of each embryo was recorded daily for 5-min up until day 18 or 19. From the time of the first recording on day 12 until the final recording made on day 18 or 19 there was a highly significant decrease in the frequency of leg motility of the curare treated embryos as compared to saline controls. For instance, on day 15 normal control embryos have an average of 20-24 movements per minute whereas curare treated animals exhibit 10-12 movements per min ($p < .0004$). Similar effects were seen at all other stages. Although curare treated embryos show small amounts of ankylosis (e.g. ankle joint extension was reduced by 10-15%) and slightly lower leg weights (i.e. .79g vs .93g) as compared to controls, there was no correlation between these deficits and the motility deficits. Histological examination of the leg musculature and lumbar spinal cord, including cell counts of motoneurons, revealed no differences between experimentals and controls. Ultrastructural and biochemical studies of these preparations are currently in progress. Whereas neostigmine (3 µl of a 0.5 mg/ml solution) can antagonize the curare-reduced motility in 15-day control embryos, this effect does not occur in the "chronic" 15-day experimental embryos. This indicates that the long-term motility deficits in the "chronic" group can not be explained by the persistence of low amounts of unmetabolized curare at the neuromuscular junction.

These findings suggest: (a) that previous reports that a short, 24 hr period of total paralysis in the chick was both a necessary and sufficient condition for the induction of joint deficits may be incorrect. Those studies did not consider the chronic subsequent partial reduction of motility which accompanies such treatment, as demonstrated here; and (b) that a short-term loss of function at the neuromuscular junction during embryogenesis may seriously impair the later ability of this structure to function normally.

286 FUNCTIONAL RECOVERY IN THE NEONATE AFTER LATERAL OLFACTORY TRACT SECTION. Rochelle Small, Dept. Biopsych., CUNY and Dept. Anat., Mt. Sinai Sch. of Med., New York, NY 10029

Hamster pups normally show a sharp decline in thermotaxis at day 8, which is prevented by bilateral or unilateral olfactory bulbectomy (see abstract by Leonard). Following neonatal transection of the lateral olfactory tract, Devor (1976) has described rearrangements in the adult olfactory bulb projection field which are related to the sparing of an adult olfactory-dependent behavior. Since thermotaxis is a sensitive behavioral indicator of olfactory function, it was used in the present study to assess the onset of functional recovery in the neonate, after early tract sections. The time course of anatomical reorganization was examined by autoradiography and degeneration techniques and related to the pattern of thermotaxis shown by individual pups.

Pups were given daily 2 min. tests on a thermal gradient from days 3 through 15. Following testing on day 5, pups were assigned to one of the following treatment groups: ULOT - unilateral section of the lateral olfactory tract (N=69); UOB - unilateral aspiration of the olfactory bulbs (N=28); C - control treatments of either bilateral aspiration of frontal cortex, sham operates or untreated normals (N=25).

Through day 9, ULOT and UOB pups were indistinguishable, showing a persistent heat preference. On day 10, the ULOT group differed from both UOB and C groups ($p < .05$ Kolmogorov-Smirov test). The normal pattern of thermotaxis was seen in ULOT pups from day 11 on, suggesting that the olfactory system had sufficiently reorganized for it to resume its role in inhibiting thermotaxis. Heat preferences of UOB pups decreased through day 15 but remained significantly higher than controls throughout testing ($p < .01$).

Variability measures indicated that ULOT was not a homogeneous group. Summing thermal scores before and after day 10 provided an initial and terminal index of heat preference which was used to separate the ULOT group into: 1)Recoverers-initial heat preference index comparable to initial UOB index and terminal index comparable to controls; 2)Nonrecoverers-initial and terminal indices similar to UOBs; 3)Pups showing no effect - initial and terminal indices similar to controls.

At completion of testing, all lesions were confirmed histologically without knowledge of behavior. The projection field of the olfactory bulbs was examined in ULOT pups by injecting the bulbs with ^{3}H-Leucine and sacrificing at 18 hrs. or aspirating the bulbs and sacrificing at 48-72 hrs. To follow the time course of reorganization, some ULOT pups were injected at 2, 7, 12 and 17 days after tract section.

Fibers extending distal to the cut and innervating the olfactory tubercle have been found in the recoverers examined to date. No such fibers have been seen in nonrecoverers, who in general suffered more extensive damage to the olfactory stalk. Pups showing no effect proved to have partial lesions.

This work suggests that the anatomical reorganization occurring after early tract section is functional in the neonate. Work is currently being done to determine if the fibers seen distal to the cut are transected axons that have regenerated, or normal fibers that had not reached the level of the cut on day 5. (Supported by Grant NS12458).

287 CHEMOSENSITIVITY OF EMBRYONIC AMPHIBIAN NEURONS IN VIVO AND IN VITRO. Nicholas C. Spitzer.Dept. Biol.,Univ. Calif. San Diego, La Jolla,CA 92093

The development of electrical excitability has been studied in Rohon-Beard (R-B) neurons in vivo (Spitzer & Baccaglini, Br. Res., in press) and in a larger group of embryonic amphibian nerve cells dispersed in culture (Spitzer & Lamborghini, P.N.A.S., in press). However it was not possible to distinguish R-B cells from other neurons in vitro, by anatomical or physiological cues. I have investigated the chemosensitivity of R-B cells in vivo, to learn if the presence of specific receptors provides a basis for identification of the cells in vitro, and to establish a baseline with which developmental changes can be compared.

Xenopus embryos were dissected to expose the dorsal spinal cord and perfused with Ringer's solution. R-B cells were impaled with intracellular microelectrodes and their response to bath application (10-50 sec duration) of various putative neurotransmitters was examined. R-B neurons are sensitive to γ-amino butyric acid (GABA)(10^{-4}M) from Nieuwkoop & Faber (NF) stage 23 (24 hr) to stage 49 (11 days). The cells are depolarized by as much as 15 mV from resting potentials of -90 mV. The average rise time of the response is 5 sec, and the average duration is 30 sec. The duration of the response is independent of the duration of bath application, indicating the existence of desensitization. In contrast, these cells showed no response to glycine (GLY), glutamate (GLU), norepinephrine (NE), serotonin (5HT) and dopamine (DA)(all 10^{-4}M).

Cultures were prepared from cells dissociated from neural plate at NF 15, and nerve cells examined as above. In cultures 24 hr (NF 32 equivalent) to 48 hr (NF 40) old, two classes of sensitive neurons were observed. The first is depolarized by GABA (10^{-4}M), and the response is as large as 15 mV from resting potentials of -75 mV; the rise time and duration are also similar to those of R-B cells in vivo. These cells are not affected by GLY, GLU, NE, 5HT, DA, or acetylcholine (ACh). The second class is hyperpolarized by GABA and GLY, depolarized by GLU, and unaffected by NE, 5HT, DA, and ACh (all 10^{-4}M). Cells are hyperpolarized by about 10 mV and depolarized by a similar amount from resting potentials of -65 mV. Rise times and durations are similar to those of the potential changes of R-B cells in vivo. The cultured neurons are not contiguous with other cells, thus eliminating the possibility that the responses are synaptically mediated. The different sensitivities of the cultured neurons suggest that there are two populations of nerve cells in vitro. One of these closely resembles the R-B cells in vivo.

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288 DEVELOPMENTAL PATTERNS OF GLYCOLYTIC ENZYMES IN REGENERATING RAT SKELETAL MUSCLE. Kenneth R. Wagner*, Bruce M. Carlson* and Stephen R. Max. Depts. Neurol. & Peds., Univ. Md., Sch. Med., Baltimore, MD. 21201 and Dept. Anat., Univ. Mich. Sch. Med., Ann Arbor, MI. 48104.

Biochemical study of regenerating skeletal muscle in vivo has not been feasible due to lack of a suitable animal model. Recently, it has been demonstrated that pre-treatment of the rat extensor digitorum longus (EDL) muscle with the myotoxic local anesthetic Marcaine followed by free grafting of the muscle causes essentially complete degeneration followed by regeneration. The resultant muscle contains myofibers that are newly regenerated and contains virtually no original muscle fibers. We have used this system to investigate the developmental patterns of a number of enzymes associated with glycolysis. For preparation of regenerating muscles, the EDL muscles were removed, injected with Marcaine (0.75% - Winthrop) plus hyaluronidase in 0.9% NaCl, and then soaked in Marcaine solution for 10 min. The muscles were then orthotopically grafted into their own beds. The contralateral EDL muscles were not touched and served as normal controls. At 1, 2, 3, 4, 5, 7, 11, 36 and 69 days after grafting, regenerating and control muscles were excised and homogenized. Enzyme assays were performed on 18,000 g. supernatants prepared from the homogenates. The data are given in the following table:

Enzyme	Days after Grafting								
	1	2	3	4	5	7	11	36	69
HK	26*	54	109	135	147	147	148	81	132
G-6-PDH	166	148	295	295	330	698	367	266	62
Phos.	5	9	8	2	1	13	8	9	20
PFK	3	6	---	5	4	3	6	43	62
α-GPDH	2	14	7	10	17	9	13	39	40
LDH	13	13	22	25	31	45	35	37	70
PK	6	15	18	21	36	32	21	35	73
CK	7	9	8	14	18	29	46	65	127
AK	8	8	9	15	17	25	47	77	77

*All values are % control.

The enzyme activities fell into three groups with respect to their developmental patterns: 1) initial increase followed by return to control levels: hexokinase, glucose-6-phosphate dehydrogenase; 2) initial reduction followed by very slow recovery: phosphorylase, phosphofructokinase, α-glycerophosphate dehydrogenase; 3) initial decrease followed by more rapid recovery: pyruvate kinase, lactate dehydrogenase, creatine kinase and adenylate kinase.

The following conclusions are drawn from these data: 1) The sharp rises in activity of glucose-6-phosphate dehydrogenase and hexokinase are temporally correlated with the early maturation of regenerating striated muscle fibers. Development of activity of the other enzymes parallels the later maturation of the regenerating muscle fibers. 2) Glycolysis is probably not a major source of metabolic energy for support of early stages of regeneration. 3) The increased hexokinase and glucose-6-phosphate dehydrogenase reflect enhanced hexosemonophosphate shunt activity, but some contributions from scavenger cells cannot be ruled out. 4) The course of development of these enzymes during muscle regeneration is different from that seen in developmental studies of pre- and neonatal rats and in tissue culture research. Thus, regeneration may not recapitulate the embryogenesis of muscle with respect to biochemical maturation. 5) The Marcaine-treated, freely-grafted EDL represents a useful system for the biochemical study of muscle regeneration. (Supported by grants from N.I.H., the Muscular Dystrophy Assn., and the Bressler Fund.)

289 DEVELOPMENTAL CHANGES IN NEUROLEPTIC-INDUCED CATALEPSY. Luis A. Baez, Dorothy K. Burt*, James Granneman* and Craig Shanklin*. Dept. Psychol., Southern Illinois U., Carbondale, IL 62901.

The cataleptic response elicited by chlorpromazine (CPZ) and by the specific dopaminergic antagonists spiperone (spiroperidol, SPI) and haloperidol (HPD), was investigated in Long-Evans rats 10, 15 and 20 days old. Catalepsy and righting responses were evaluated at 30, 60 and 300 minutes after administration of saline or various doses of the neuroleptics. SPI and HPD were markedly less effective in producing catalepsy at 15 days of age, than in either younger or older rats, although the time-course of the effect was similar accross ages. In contrast, sensitivity to CPZ declined consistently with increasing age. Catalepsy produced by CPZ was associated with depression of righting responses, whereas both butyrophenones were highly specific cataleptogens and did not impair righting at any dose. In so far as catalepsy is related to blockade of dopaminergic synapses, these results suggest that major changes in dopaminergic function are taking place in the period between 10 and 20 days postnatally. Synapse formation (Hattori & McGeer, Exp. Neurol., 1973) and maturation of cholinergic interneurons (Butcher & Hodge, Brain Res., 1976) in the neostriatum are taking place at a rapid rate during this period. The cataleptic and antiamphetamine actions of the cholinomimetic pilocarpine also become elicitable during the third postnatal week (Baez et al., Eur. J. Pharmac., 1976; Fibiger et al., JCPP, 1970). It is possible that the initial decrease in sensitivity to SPI and HPD is due to increased activity in dopaminergic synapses. Development of antagonistic cholinergic neurons could account for the reversal of this effect by 20 days of age.

290 GENES INFLUENCE MATURATION OF PERIPHERAL NERVE FUNCTION IN MICE. Astrid B. Boening* and Joseph P. Hegmann. Dept. Zool., Univ. of Iowa, Iowa City, IA. 52242.

Fifteen generations of within-family bidirectional selection for 45-day caudal nerve conduction velocity (CNCV) in mice produced high line (HL) animals with average CNCV of 21.3 m/sec, low line mice (LL) with average CNCV of 18.0 m/sec and control animals (CL) whose mean CNCV was 20.8 m/sec. All mice show maturational increases in CNCV with age, but the lines remain divergent at 70 and 120 days even though selection was based solely on 45 day conduction velocity. Divergence is maintained because HL mice show largest increase from 45 to 70 days while LL animals increase more from 70 to 120 days. Thus, we conclude that genetic correlations between function at different ages (indicated by the maintained separation between lines at ages beyond 45 days) are due, in part, to genetic variance for maturation of conduction velocity. Patterns of body weight change do not differ between lines (though maturation is observed) so genetic variance for change in nervous system function seems to be independent of gene influence on morphological development.

291 MECHANISMS OF ADHESION AMONG CELLS FROM NEURAL TISSUES OF THE CHICK EMBRYO. Robert Brackenbury*, Jean-Paul Thiery*, Urs Rutishauser* and Gerald M. Edelman. The Rockefeller University, New York, NY 10021.

Using a quantitative assay which measures rapid binding between pairs of cells, we have found that the adhesiveness of dissociated retinal and brain cells varied as a function of developmental age. The period of maximum binding was different for cells from the two tissues, yet brain cells from 6-day embryos adhered as well to retinal cells from 9-day embryos as each cell type did to itself. Two proteins released by retinal cells in culture were purified. Structural studies showed that the smaller (F2) was derived from the larger (F1) by proteolytic cleavage. Antibodies against both proteins bound to the surface of both retinal and brain cells, and anti-F2 but not anti-F1 blocked the adhesion of both cell types. Anti-F2 reacted weakly with F1, indicating that the determinants presumed to be involved in cell-cell binding are masked in F1. Both antibodies specifically precipitated several iodinated retinal cell surface proteins of different molecular weights. These results will be discussed in terms of a model in which proteolytic cleavage of inactive F1-like cell surface molecules gives rise to F2-like molecules that participate in forming cell-cell bridges.

292 BEHAVIORAL EFFECTS OF DAMAGE TO AUDITORY PATHWAYS IN KITTENS. K.A. Brown*, J.S. Buchwald, J.A. Schwafel*, K.R. Kanegawa*, and J.R. Johnson*, Dept. Physiol., Mental Retard. Res. Ctr., BRI, UCLA, CA 90024.

Five groups of kittens were prepared at 30 days of age with bilateral 1) cochlear destruction (Deaf), 2) auditory meatus obstruction (Restricted), 3) midbrain reticular lesions (RF), 4) inferior collicular brachia lesions (IC), and 5) sham or no operation (Normal). Subsequently, subjects were tested in auditory-behavioral situations during development and as adults. No marked deficits in auditorally cued behavior were observed consistently in any group except for the Deaf subjects. All other groups showed comparable passive avoidance and auditory intensity discrimination, pattern discrimination, and sound localization as indicated by response acquisition in T-maze and shuttle box. Differences between groups emerged in measures of arousal, stimulus reactivity, or distractibility but were more subtle than reported for adult-lesioned subjects. Vocal responses of Deaf and IC subjects, however, showed immediate and persistent postoperative changes. Auditory evoked potential recordings from medial geniculate and intralaminar thalamic nuclei of the adult subjects indicate short-latency (10-15 msec) components in both the lateral, classical and medial, "extralemniscal" auditory pathways; these alternative pathways may underlie retention of auditory processing capabilities in infant-lesioned subjects. (Supported by NIH Grant MH24344.)

293 NEURONAL ABNORMALITIES IN NEOCORTEX OF JIMPY MICE: A GOLGI ANALYSIS. F.K. Butler* & D.A. Kristt, Johns Hopkins University School of Medicine, Division of Neuropathology, Baltimore, Maryland 21205

In the homozygous mutant mouse, jimpy, there is a severe defect in CNS myelination. The defect in myelination is accompanied by impaired axonic growth. We examined the effect of such impaired axonic growth on dendritic development in jimpy somesthetic cortex. The possibility of abnormalities in dendritic development is suggested by previous work showing that neocortical neurons deprived of specific afferents during development exhibit altered dendritic orientation. Jimpy and heterozygous (control) mice, age 11-13 days, were analyzed using a rapid Golgi method. At this age cortical myelination normally begins and the impaired axonal function and growth should be manifest. In jimpy mice many superficial pyramidal neurons exhibited striking mal-orientation of their apical dendrite, but qualitatively were otherwise normally developed. Occasionally, immature neurons with large (25 μm dia.), globular somata were also encountered. The altered geometry of apical dendrites was quantified by measuring the angle between the apical dendrite and a radial line (perpendicular to pia) passing through the center of the soma of randomly selected pyramids. Mean deviation for jimpy apical dendrites is 13° ± 25°; for controls, 2° ± 6°. The incidence of neurons with mal-oriented dendrites is 29.8% in jimpy; 6.2% in controls. The somata of these mal-oriented dendrites were properly oriented and were located in the superficial one-quarter of cortex (mean depth: 222 μm). We conclude that in jimpy mouse neocortex the geometry of apical dendrites of pyramids from layers II and III is abnormal. These abnormalities are believed to be the consequence of impaired growth of jimpy axons resulting in abnormal innervation to growing dendrites.

294 EARLY APPEARANCE OF ^{3}H-TdR LABELLED DEGENERATING CELLS IN EMBRYONIC CHICK DORSAL ROOT GANGLIA. Virginia McMillan Carr and Sidney B. Simpson, Jr.* Dept. Biol. Sci., Northwestern Uni., Evanston, Ill. 60201.

This study was undertaken to determine the length of time required between uptake of ^{3}H-TdR and the appearance of degenerating cells in the dorsal root ganglia (DRG's) of 5½ day chick embryos. This age has been shown previously to represent the peak in normally occurring DRG degenerative activity. By 2 hours after delivery of the ^{3}H-TdR (25 μCi) to the blastoderms of the embryos, as many as 15% of the degenerating cells were found to be labelled. No labelled degenerating cells were seen at one hour. The percentage of degenerating cells that were labelled continued to rise for the next 12 to 18 hours. During this time the percentages of degenerating cells labelled in cervical and brachial ganglia were almost the same despite much greater cervical degenerative activity. After this time the brachial values continued increasing to a peak (~65%) while the cervical values declined. Preliminary results suggest a similar relationship may exist between ipsi- and contralateral brachial ganglia following unilateral wing bud amputation at 2½ days as that between normal cervical and brachial ganglia.

Two hours is too soon after delivery of ^{3}H-TdR for completion of the mitotic cycle by labelled neuronal precursors and for fiber outgrowth and establishment of peripheral contact by daughter neurons prior to degeneration. Thus, the results suggest that degeneration of some ganglionic cells may not be due directly to establishment of faulty peripheral contacts. The similar proportions of degenerating cells labelled in cervical and brachial ganglia suggests, instead, a more indirect peripheral control of degeneration, perhaps via other cells that are themselves degenerating due to faulty peripheral contacts.

295 EFFECT OF DESTRUCTION OF THE POST-GANGLIONIC SYMPATHETIC NEURONS IN NEONATAL RATS ON DEVELOPMENT OF CHOLINE ACETYLTRANSFERASE AND SURVIVAL OF PREGANGLIONIC CHOLINERGIC NEURONS. M.T. Caserta*, E.M. Johnson, Jr., and L.L. Ross. Depts. of Anatomy and Pharmacology, Medical College of Penna., Phila., Penna. 19129

The peripheral sympathetic nervous system of neonatal rats was destroyed by administering guanethidine (50mg/kg/day, 5 day/week for 3 weeks). The tyrosine hydroxylase activity in the superior cervical ganglion (SCG) was reduced to undetectable levels by 10 days. Light microscopic examination of the ganglia beginning 10 days after starting treatment showed almost complete cell loss. Choline acetyltransferase (CAT) in the SCG of treated animals failed to attain normal adult values, but remained at the level of 10 day old animals. Adrenal CAT levels, on the other hand, developed normally in sympathectomized and saline-treated control animals (the adrenal medulla is not destroyed by guanethidine). Accompanying the failure of normal increase in the CAT levels of the SCG is a loss of cells in the intermediolateral nucleus (IML) of the thoracic spinal cord. Cell counts in this nucleus at the thoracic (T1) segment showed 24% decrease ($P < .001$) in 7 month-old sympathectomized rats. Preliminary examination of the spinal cords from 2 to 8 week old animals indicated that most of the cell death occurs between 6 and 8 weeks of age. These data support the hypothesis that during the developmental phase the survival of the preganglionic neuron is dependent upon the presence of its end-organ, the post-ganglionic neuron.

(Supported by the National Foundation, March of Dimes, NIH #HL16893, and #NS11364).

296 EFFECTS OF AGING ON VISUAL EVOKED RESPONSES. Gastone G. Celesia and Richard F. Daly. Dept. Neurol., St. Louis Univ., St. Louis, MO. 63104 and the Univ. of Wisc. Center for Health Sciences, Madison, WISC. 53706.

The effects of aging on visual evoked responses (VER) and critical frequency of photic driving (CFPD) was studied in 74 volunteers aged 18 to 79.

The amplitude of VER to pattern reversal stimulation did not vary with sex and/or age. The latency of the first major negative and of the first major positive deflection of the VER was significantly delayed ($p < .001$) with advancing age. This increase of latency probably reflects a slowing of conduction velocity in the optic nerve and/or optic pathways. CFPD, defined as the highest frequency of photic driving response expressed in flashes per second, showed an inverse correlation with age decreasing in older subjects. CFPD is the electrophysiological counterpart of critical flicker fusion which is known to decrease with advancing age.

These data support the concept that aging influences the functions of specialized sensory systems.

297 **EFFECTS** OF MALNUTRITION ON THE DEVELOPMENT OF THE CEREBRAL CORTEX OF THE RAT. Ana G. Colmenares. Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

The effects of malnutrition on the developing somatosensory cortex of the rat were studied with anatomical methods. Pregnant Charles River CD rats were given either a 24% (control) or 8% (experimental) protein diet (Shoemaker and Wurtman, 1971), starting at day 10 of gestation and continuing until 20 days after birth. Observations were made on tissue from animals 20 and 40 days old fixed by vascular perfusion of aldehydes and embedded in Araldite.

The dimensions of the cerebral hemispheres in 20 day experimental animals were significantly decreased compared with controls: length, 10% less, $P<.001$; width, 8% less, $P<.001$; height, 3% less, $P<.05$. Differences in length and width were also statistically significant ($P<.01$, $P<.001$) at 40 days but were not as great as at 20 days. The cerebral cortex at 20 days was 13% thinner in the malnourished animals ($P<.01$). Analysis of covariance showed that experimental animals had a significantly greater increase in dimensions between 20 and 40 days than controls. Volume fractional analysis by point counting of tangential 1 μm sections through layers II to IV of the 20 day old animals indicates a difference between experimentals and controls in the proportion of tissue occupied by neuropil. This difference is greatest in the upper part of layer II ($P<.01$), is less in the lower half of layer II ($P<.05$) and is not statistically significant in layer IV.

298 THE EFFECT OF CHRONIC PHENOBARBITAL ADMINISTRATION UPON BRAIN GROWTH OF ARTIFICIALLY REARED RATS. Jaime Diaz, Gay Bailey* and Richard J. Schain. Dept. of Psychiatry, Neurology & Pediatrics, Neuropsychiatric Institute, UCLA, Los Angeles, CA 90024.

In spite of the widespread clinical use of phenobarbital in human infants, there has been very little experimental information about the effect of this drug on the developing brain. Initial studies indicated that chronic phenobarbital retards brain growth in a manner that appeared to be independent of reduced food intake. The present study was designed to test the effect of chronic phenobarbital on brain growth of the developing rat under conditions of controlled nutrient intake. Chronic intragastric cannulas were installed in 4 day old male Wistar rat pups, according to a technique recently described by Hall (Science, 190, 1313, 1975). Half of the animals were given daily subcutaneous injections of phenobarbital (0.06mg/g) and the other half were given the inert vehicle. The injections were started on day 5 and continued until the animals were 18 days old. Control and phenobarbital animals received equal amounts of milk via intragastric cannulas. On day 19, the animals were weighed, tested in an open field, and sacrificed. No frank difference in motor activity between the two groups was observed at that time. Brain and body weights are given in the table below:

	Body Wt. (gm)	% Diff.	Total Brain Wt. (gm)	% Diff.	Cerebellum Wt.(gm)	% Diff.
Control	35.6	—	1.234	—	0.162	—
Phenobarbital	34.7	-3	1.100	-11	0.151	-7
Significance		NS		$p<0.001$		$p<0.05$

These data indicate that chronic phenobarbital administration during the preweaning period in the rat significantly retards brain growth independently of nutritional intake.

299 TARGET ORGAN REGULATION OF SYMPATHETIC NEURON DEVELOPMENT. Mark D. Dibner, Catherine Mytilineou, and Ira B. Black. Lab. of Develop. Neurol., Cornell Med. Ctr., New York, NY 10021.

The role of target organs in the morphological and biochemical development of sympathetic neurons was examined in the neonatal rat. The superior cervical ganglion (SCG) and its end organs, the salivary glands and iris were employed as a model system. Unilateral sialectomy and iridectomy prevented the normal developmental increase in ipsilateral ganglion tyrosine hydroxylase (T-OH) activity, a marker for adrenergic maturation. Enzyme activity remained depressed by approximately 30 percent for at least 6 months, the longest time tested.

Ganglion morphometry was performed to investigate the basis of the abnormal biochemical ontogeny. Target organ removal significantly decreased the number of adrenergic neurons in the SCG by approximately 30 percent. Total ganglion volume was reduced in a parallel fashion. Thus, end organ extirpation may prevent the biochemical maturation of the SCG by decreasing adrenergic neuron survival.

Sialectomy without iridectomy prevented the normal postnatal increase in ganglion T-OH activity, but did not alter iris activity. These observations suggest that target removal prevents the development of only those neurons destined to innervate that organ.

In addition to preventing normal adrenergic neuron ontogeny, target extirpation also prevented the normal development of presynaptic choline acetyltransferase activity. Presynaptic ganglion terminals may have failed to mature normally secondary to adrenergic destruction, or may have responded in some other manner to target organ extirpation.

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300 SIGNIFICANCE OF MAMMALIAN BRAIN GROWTH STAGES. H.T.Epstein, Larry B. Goldstein*,Shelley Tepper*,and Verne Woods*. Biology Dept. Brandeis Univ. Waltham,Mass. 02154

Evidence will be reviewed showing the existence of several postnatal brain growth stages in humans and in rodents. A possible meaning of these stages can be inferred from the existence of stages in mental development of humans which correspond in ages with the brain growth stages. Further, the ages of the correlated brain/mind growth stages are at the turning points of the four major Piagetian stages of intelligence development, suggesting that the brain growth stages may be the main biological bases for the Piagetian stages.A similar set of correlated brain/behavior growth stages seems to occur in rodents. In addition,the correspondence of stages of brain growth in different species may follow from our finding that the same major anatomical events occur in the last two postnatal spurts in rodents as in the first two (out of five) postnatal spurts in humans. Construction of low-variance litters,as recently achieved in our laboratory, has permitted us to study other aspects of brain growth,and the results will be described.

301 REGULATION IN THE RETINO-TECTAL SYSTEM OF EARLY CHICK EMBRYOS. T. E. Finger*, L. A. Rogers*, and W. M. Cowan. Dept. Anat. and Neurobiol., Sch. Med., Washington Univ., St. Louis, Mo. 63110

As originally described by Birge ('59), following unilateral ablations of the mesencephalic alar plate in 36-42 hour chick embryos, a single optic tectum develops which spans the midline. The half tectum on the operated side appears to develop from a rapidly proliferating ependymal layer which is derived from the adjacent intact neuroepithelium. In most cases the regenerating tectum lags behind in development in comparison to the unoperated side. However, in some cases this developmental lag has virtually disappeared by the 7th day of incubation. At this and later stages the tectal lamination on the regenerated side is normal and continuous across the midline. Occasionally there is a slight thinning of the tectal plate near the midline.

Autoradiography following unilateral intraocular injection of tritiated proline at day 18 reveals that each eye projects exclusively to the contralateral side of the unpaired tectum. The boundary between the two retinal projection zones corresponds fairly closely to the midline of the tectal plate.

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302 MATERNAL PROTEIN RESTRICTION AND BRAIN GROWTH IN THE RAT. William B. Forbes, Carl Tracy*, Oscar Resnick* and Peter J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

In continuing studies of the effects of protein malnutrition on the developing brain, we studied the growth of the brain and body in rats born of dams fed a low-protein (8% casein) diet ad lib. beginning 5 weeks prior to mating and continuing throughout gestation and lactation. Control dams were fed an isocaloric 25% casein diet. Litters were culled at birth to 8 pups and randomly fostered within dietary groups. At birth, brain weights, body weights and body lengths of pups from protein restricted dams were similar to those of controls. During the period of lactation, pups from restricted dams exhibited severely retarded body growth but only mildly retarded brain growth resulting in an elevated brain/body weight ratio. This relative macrocephaly corresponded well with the brain "growth spurt", being maximal at 10-15 days of age and declining almost completely at weaning (21 days). A similar relationship between brain weight and body length was observed. Following weaning, pups were maintained ad lib. on the diets fed their mothers. At adulthood (60+ days), brain/body weight ratios were normal in the protein restricted group. These data indicate that during the period of rapid brain growth, the rat brain is capable of nearly normal growth under conditions which severly limit the growth of the rest of the body. The relative macrocephaly observed between birth and weaning is in agreement with previously reported data of others who utilized the Widdowson and McCance paradigm (large litters) for producing malnutrition during lactation (e.g., Dobbing and Sands, 1971; Roach, et al., 1974). In contrast to these reports, however, we did not observe a relative microcephaly at adulthood, indicating that if the nutritional deficit is maintained following lactation the brain is capable of growing at a rate proportional to the growth of the body. (Supported by grant HD 06364).

303 THE EFFECT OF THE NERVE GROWTH FACTOR ANTISERUM IN THE REGENERATING OPTIC NERVE OF THE NEWT (TRITURUS VIRIDESCENS). K. A. Glaze* and J. E. Turner. Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103.

The extent, selectivity and specificity of a nerve growth factor (NGF)-central nervous system (CNS) response has not been determined although recent evidence strongly indicates that nerve growth from certain lesioned central noradrenergic pathways is responsive to NGF and its antiserum. In order to further characterize the role of NGF in the regenerating CNS of vertebrates a study was initiated to investigate the effect of anti-NGF on the regenerating optic nerve of the newt (Triturus viridescens). Animals received one 4µl injection of anti-NGF in the vitreous chamber of the eye at time of lesion. Control groups consisted of animals injected with 4µl of the vehicle (horse serum) and animals not injected at the time of lesion. Observations were made utilizing both light and electron microscope techniques. Regenerating optic nerves were prepared for study 14 days post lesion.

Light and electron microscopic studies revealed a significant reduction in numbers of ingrowing neurites of antiserum injected animals when compared with those injected with the vehicle. In contrast, there was little difference in numbers of ingrowing nerve fibers between the control groups. Glial cells in nerves of experimental animals showed marked cellular hypertrophy when compared with controls. In addition, nerves of experimental animals showed large vacuoles and a large increase of myelin figures over the number seen in the normal 14 day regenerate.

In conclusion, we feel that our studies strongly indicate that the antiserum to NGF has affected newt optic nerve regeneration at the level of the retinal ganglion cell as well as the level of the glial cell. (Supported by grants from the March of Dimes, National Society for the Prevention of Blindness and NIH grant NS-12070.)

304 ALTERATIONS OF DNA SYNTHETIC PROCESSES DUE TO STRESS DURING CEREBELLAR DEVELOPMENT. W. S. T. Griffin, R. Chanda*, and D. J. Woodward. Dept. Cell Biol., Univ. Tx. Health Sci. Center, Dallas, TX. 75235.

It is known that numerous factors such as irradiation, cytotoxic agents and malnutrition can effect cell proliferation as indicated by total DNA accumulation in the postnatally developing rat cerebellum. An aim of this study was to determine if various environmental stresses which do not directly kill cells during cerebellar development might nevertheless share some common modes of action which ultimately result in less DNA accumulation over time. In initial studies, we determined that the decrease in DNA accumulation was not due to a long term process of cell death. Single intraperitoneal (IP) injections of C-14 thymidine (0.1 µCi/g body weight) labelled cerebellar DNA at levels that remained constant over a 21 day period, indicating that no cell death or DNA turnover occurred even during an environmental stress such as long term malnutrition. We further examined the possibility of changes in uptake and incorporation of DNA precursors by subjecting 8 day old rat pups to malnutrition, temperature and handling stresses and injecting IP with C-14 thymidine. Short term stress such as decreased core temperature (16°C) and excessive handling resulted in an 11-14% ($p < 0.05$) decrease in C-14 thymidine taken into cells and incorporated into cerebellar DNA. However, in long term stress such as several days of malnutrition (rearing in litters of 20 vs 6) uptake of C-14 thymidine into cells and the amount incorporated into DNA can be increased. Thus we have shown that both short and long term conditions can alter C-14 thymidine uptake into cells and incorporation into DNA. These alterations can be considered as either deleterious reactions to or adaptive changes to stress. Supported by NIH Grants NS-13225 and GRS-05426-13.

305 PRENATAL DEVELOPMENT OF THE HUMAN INTERPEDUNCULAR NUCLEUS. Neal Halfon* and Nicholas J. Lenn. Dept. Neurol., Pediat and Carnegie Lab. of Embryol., Sch. Med., Univ. of California, Davis, CA. 95616.

Specimens in the Mall collection, Carnegie Lab. of Embryol., previously staged in the case of embryos, were studied. At embryonic stage (S) 17 the habenulopeduncular tract (HPT) is present, descending from the epithalamus. At S18 migration of cells ventrally from the ventricular zone (VZ) in the region of the pontine flexure begins and has progressed at S19. IIIrd nerve rootlets which are already present border these cells which are descending in the midline. At S20 and 21 this group of cells is 1.4 mm wide, representing 1/2 of the cross-section of the midbrain, and has a compact leading edge. As cells approach the marginal zone (MZ) their orientation changes from vertical to random. At S23 the migrating cellular mass is 2 mm wide, 1/3 of the total width, and demarcated laterally by HPT.

In the smallest fetuses, 32 to 35 mm in crown-rump length (CR) the midline migrating cells have spread laterally in MZ, giving the shape of an inverted fountain. The lateral portions of this mass are presumptive medial substantia nigra (SN). The lateral portions of SN are formed by cells migrating from lateral portions of VZ. The interpeduncular nucleus (IPN) condenses from cells remaining near the midline. At 43 mm CR IPN is distinct caudally, but rostrally is still confluent with SN. This rostral-caudal gradient is also evident in SN, and persists at 50 mm CR in both nuclei. The red nucleus is distinguishable, condensed from laterally migrating cells, after 43 mm CR. At 67 mm CR IPN is distinct throughout, located in its definitive site astride the midline in the roof of the interpeduncular fossa, and clearly receives the HPT.

This sequence is highly comparible to that described for rat IPN (Hanaway, et al., '71). These observations will be extended to non-human primates preparatory to experimental studies of this system.

306 CENTRAL REGULATION OF SYMPATHETIC NEURON DEVELOPMENT. R.W. Hamill*, E.M. Bloom* and I.B. Black. Dept. Neurol., Lab. of Develop. Neurol., Cornell Univ. Med. College, New York, NY 10021.

The sixth lumbar (L-6) ganglion has been employed as a model system to study the central regulation of peripheral sympathetic neuron development. During the course of postnatal ganglion ontogeny, presynaptic choline acetyltransferase (ChAc) activity increased 100-fold and postsynaptic tyrosine hydroxylase (T-OH) activity increased 60-fold. Total ganglion protein rose 10 times. Transection of the spinal cord at the fifth thoracic (T-5) segment in neonatal rats prevented the normal developmental increase in ganglion ChAc and T-OH activities. However, spinal transection did not alter the ontogeny of ChAc or T-OH in the superior cervical ganglion. This ganglion derives its innervation from spinal segments rostral to the surgical lesion. Thus, spinal transection prevented the normal maturation of sympathetic neurons distal to, but not proximal to the lesion. The effect of transection on the L-6 ganglion persisted for at least one month, the longest time tested. Our observations suggest that trans-synaptic regulation of adrenergic maturation in the periphery is governed by suprasegmental mechanisms in the central nervous system (CNS). Moreover, the development of presynaptic cholinergic neurons is also regulated by higher centers in the CNS.

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307 CEREBRAL BLOOD FLOW AND OXIDATIVE METABOLISM IN THE NEWBORN DOG. M.J. Hernández, R.W. Brennan, R.C. Vannucci and G.S. Bowman (SPON:I.S. Zagon). Hershey Medical Center, Hershey, PA 17033.

We have applied the Kety-Schmidt method modified for $Xenon^{133}$ to define CBF characteristics in the newborn dog, an experimental species whose cerebral maturation at birth approximates that of man. Cerebral cortical glycolytic and high energy metabolites were measured by standard freezing and assay techniques in the same model. Mongrel dogs 1-12 days of age were paralyzed and passively ventilated with 70% N_2O and 30% O_2. CBF was derived by analysis of paired serial 20 ul samples of arterial and of cerebral venous blood from superior sagittal sinus. At normocapnia ($PaCO_2$ = 35.7 ± 3.1 mm Hg) and normotension (MABP = 57 + 10 mm Hg) CBF averaged 30 + 4 ml/min/100 gm, and $CMRO_2$ was 1.7 ± 0.4 ml/min/100 gm. CBF responsiveness to graded hypercarbia was 1.0 ml/min/100 gm per mm Hg change in arterial PCO_2. Cerebral glycolytic metabolites (glucose, pyruvate, lactate) and high energy reserves (ATP, ADP, phosphocreatine) were similar to those reported for adult animals.[1] Our results suggest that CBF and $CMRO_2$ are significantly lower in the neonate than in the adult[2], but that oxygen delivery under normal conditions is adequate for the metabolic needs of the immature nervous system.

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308 ORGANIC BRAIN SYNDROME, EDUCATION AND COGITIVE FUNCTION IN SENESCENCE. Nancy M. Hilbert, Steven H. Zarit*, and Robert L. Kahn*, Dept. of Psychiatry, Univ. of Chicago, IL 60637.

In a study of factors affecting intellectual performance and brain damage in the elderly, the relation of two measures of mental status to cognitive test scores and years of education has been evaluated. The sample consists of 153 consecutive persons 50 years of age and up referred for psychiatric evaluation. Ss were given 2 measures of mental status, the Mental Status Questionnaire (MSQ) and the Face-Hand Test (FHT), which have previously been validated on clinical ratings of organic brain syndrome. Intellectual performance was evaluated with 8 tests of learning and memory, such as Paired-Associates and Babcock Story Recall. Scores on the mental status tests have been categorized as: 1) no errors; 2) non-significant errors (which do not reach established criteria for indicating presence of brain dysfunction); 3) moderate dysfunction; and 4) severe dysfunction. Scores of intellectual tests were then related to each mental status test, to a combined measure of both procedures, and to education. The findings indicate that performance on all intellectual measures showed a consistent decline with increasing errors on the MSQ, the FHT and on the combined mental status score. This decline was manifested by persons at each education level. Both non-significant errors on the mental status tests and instances where only one test was positive for brain dysfunction were associated with lowered intellectual performance. Among those with brain impairment, intellectual performance was still related to level of education. Thus, college educated persons with organic brain syndromes scored higher on memory tests than Ss with minimal education and no brain dysfunction. These results suggest that in assessing behavioral problems of older persons, the interaction of brain function, education and intellectual performance must be considered.

309 AGING IN THE RAT OLFACTORY BULB. James W. Hinds and Nancy A. McNelly*. Dept. Anatomy, Boston Univ. School of Medicine, Boston, MA. 02118.

A quantitative study of the rat olfactory bulb during aging was carried out by directly measuring or calculating the following parameters at 3, 12, 24, 27, and 30 months: the volume of the glomerular, external plexiform, and internal granular layers, a relative measure of the size of the olfactory nerve layer, the mean volume of mitral cell nuclei and perikarya, a relative measure of the mean volume of the mitral cell dendritic tree as well as its total length and mean cross-sectional area of its constituent dendrites, and number of mitral cells. In addition, measurements of the size and number of mitral cells in the accessory olfactory bulb (AOB) were performed. Data were analyzed statistically.

From 3 to 24 months a linear increase of approximately 50% occurs in all layers of the olfactory bulb. During this time the mean perikaryal volume and dendritic volume of mitral cells increases, also in a linear fashion, approximately 100%. No significant change occurs in the number of mitral cells. From 24 to 30 months a significant decrease occurs in the volume of the layers. Although the total volume of mitral cell dendritic trees decreases slightly from 24 to 27 months, the volume of individual mitral cell dendritic trees, as well as perikaryal and nuclear size, increases sharply, apparently in compensation for a sharp decrease in the number of mitral cells which occurs at this time. From 27 to 30 months no further decrease in mitral cell number occurs, but the size of mitral cell perikarya, and especially dendritic trees, decreases sharply.

The coordinated increase in olfactory bulb size from 3 to 24 months appears to be a continuation into adult life of earlier postnatal increases. The atrophy from 24 to 30 months appears not to be associated with peripheral rhinitis, since atrophy also occurs in the AOB, which is innervated by an organ (vomeronasal) not normally subject to rhinitis. (Supported by USPHS grant HD-05796.)

310 A GOLGI STUDY OF THE EARLY POSTNATAL DEVELOPMENT OF RAT VISUAL CORTEX. Janice M. Juraska and Eva Fifkova. Dept. Psych., Univ. Colo., Boulder, CO 80309.

Although functional plasticity has been extensively studied in the visual cortex of rats, no systematic data on this area's development are available. In the present study, the visual cortex of infant hooded rats ages 1, 3, 5, 7 and 10 days was stained by the rapid Golgi method. The cortex was divided into the superficial layers, composed of the marginal zone and cortical plate in days 1-5 and layers I-IV in days 7 and 10, and the deep layers corresponding to the intermediate zone in days 1-5 and layers V and VI in days 7 and 10. Pyramidal neurons composed the vast majority of neurons stained. A very small number of neurons from days 3-10 were non-pyramidal, probably stellates. The pyramidal neurons had apical shafts already present at day 1 as well as the beginning of the apical terminal arch. The superficial pyramidal cells had an average of one order of branches less than the deep pyramidals for days 1-5. This difference decreased by day 7 and was not found in day 10 where both groups had branches through the fifth order. By day 10, Nissl preparations revealed an adult layering pattern. Dendrites at all ages exhibited varicosities which were especially prominent on the very thin basilar and oblique dendrites of the earlier ages. Some thin filamentous processes were found on dendrites and cell bodies in days 1-5, being the most numerous in day 5. A very small number of dendritic spines appeared on basilar, oblique and apical dendrites in days 5 and 7. By day 10 the number of spines sizably increased. Axons were seen even in very immature cells on day 1, and by day 3 axon collaterals were noted. Thus by day 10 the layering and dendritic branching pattern of pyramidal neurons is qualitatively very similar to the adult, while the spines are still not as numerous as in the adult picture.

311 POSTNATAL DEVELOPMENT OF VISUAL ACUITY, THE RETINA AND STRIATE CORTEX IN THE SQUIRREL MONKEY. B. Kaack*, Delta Regional Primate Center, Covington, La. 70433 (SPON: A.A. Gerall), Psychology Department, Tulane University, New Orleans, Louisiana 70118.

Increasing interest has been directed toward the use of the squirrel monkey as a primate model for the study of brain mechanisms, behavior and the effects on the brain of such environmental hazards as radiation, drugs and malnutrition. Visually guided behavior is one of the most prominent characteristics of the squirrel monkey as it is in all diurnal primates, including man. Considerable research has been reported on specific visual functions in relation to the organization of the retina and the visual pathways and centers of the brain in adult squirrel monkeys. Only a few studies have been reported on the postnatal development of learning, sensory-motor capacity and the development of the brain in the squirrel monkey. The aims of this study were to examine the postnatal development of visual acuity in relation to the organization of the retina and striate cortex of the squirrel monkey. The subjects for this study included 10 infants ranging from birth to 12 months. Minimum separable visual acuity determined by optikenetic nystagmus was 36 minutes of arc, 5-7 days after birth. Adult acuity thresholds of 1 minute of arc were established with behavioral discrimination tests by the end of the first year after birth. Histological examinations indicated advanced development of cones, rods, bipolar and ganglion cell layers, a foveal depression and a characteristic adult macula in the retina by the end of the second month after birth. By 5 months after birth, there was relatively complete development of the striate cortex in terms of isocortical stratification, cell packing density and other features of the cytoarchitectural organization of the striate cortex. (Supported in part by NIH grants HD/NS 09942-01 and RR00164-14).

312 IMPAIRED SYNAPTIC POTENTIATION IN THE HIPPOCAMPUS OF AGED, RETENTION-DEFICIENT RATS. Philip W. Landfield, James L. McGaugh, and Gary Lynch. Dept. Psychobiology, Univ. of Calif., Irvine, 92717.

A series of *in vitro* and *in vivo* neurophysiological experiments were conducted on the hippocampal Schaffer collateral system of aged and young Fischer rats. The aged rats have previously been found to exhibit retention performance deficits. No age-related differences were found between extracellular synaptic responses of aged and young animals as long as very low frequency (0.3 Hz) control stimulation of the Schaffer collaterals was employed. Moreover, spontaneous single unit activity did not appear to be different in old and young animals. With higher frequency repetitive stimulation young synapses showed typical patterns of frequency and posttetanic potentiation. However, repetitive stimulation in aged animals induced synaptic depression following a brief initial potentiation. The same patterns were found in both the intact anesthetized preparation and in the hippocampal slice preparation. These data are interpreted to suggest possible age-related weaknesses in transmitter function and they suggest possible future directions for chemical analysis of brain aging. The data seem to raise the possibility that the deficits in synaptic plasticity are related to deficits in behavioral plasticity.

313 AVOIDANCE ACQUISITION IN DEVELOPMENTALLY PROTEIN MALNOURISHED ADULT RATS. J. Patrick Leahy*, Warren C. Stern, Peter J. Morgane and Oscar Resnick*, (SPON: W.L. McFarland). Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

Developmentally protein malnourished and normally nourished adult female rats were subjected to avoidance acquisition procedures in an attempt to determine the effects of this type of pre- and postnatal dietary regimen on learning of aversively motivated tasks in adulthood. Female rats of the LP (low protein, 8% casein) and NP (normal protein, 25% casein) groups were 110 days old at the time of training on one-way active avoidance (criterion: 5 successive avoidances) and extinction (+21 hr.). No differences were found between LP and NP groups for trials to criterion on acquisition (X + S.E.: 9.20 ± 1.08 vs 10.71 ± 0.87, respectively) or extinction (11.87 ± 3.27 vs 17.17 ± 2.69, respectively). At 145 days of age these animals were subjected to passive avoidance training. After fluid deprivation and stabilization of drinking latency and rate in the experimental chamber (3 days for 30 min. per day) drinking attempts were punished by a 1 ma. shock through the spout. The number of drinking attempts by the LP group (9.50 ± 1.52) did not differ from those of the NP group (8.13 ± 2.10). Other groups of LP and NP females were tested on two-way active avoidance acquisition (100 massed trials) at 110 days of age. The NP group was superior to the LP group in acquisition rate and terminal performance (83.33 ± 7.30% vs 40.00 ± 8.49% avoidance for the last block of 10 trials), indicating that task complexity may be a factor in the poor performance of the LP group. Studies are underway to evaluate possible endocrine contributions to the learning deficits seen in LP rats.

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314 MOSAIC OF THE MOUSE OTOCYST : AN IN VITRO STUDY OF THE SITE OF ORIGIN OF THE EMBRYO INNER EAR SENSORY STRUCTURES. Cheuk W. Li. Dept.Otolaryngology Albert Einstein College of Medicine, Bronx, New York 10461.

Approximately 600 mouse otocysts of gestation days 11, 12 and 13 were dissected into six anatomical groups of dorsal, ventral, anterior, posterior, medial and lateral halves. The statoacoustic ganglion complex was included in the ventral, anterior and medial halves of the otocysts. The whole endolymphatic projection was included in the dorsal and medial halves of the otocysts, and the anterior and posterior portions shared half of the endolymphatic projection. Every fifth specimen of each anatomical group was histologically fixed as control. Each experimental group was then placed in organ culture dishes and then maintained in vitro at 34.5°C for a period of ten days. At the end of the experiment, the specimens were fixed and processed by Harris hematoxlin and eosin method. Histodifferentiation of sensory structures of each specimen was determined by microscopic analysis. Three semicircular canals and their associated cristae were found to develop in the dorsal and lateral halves of the otocysts. Only one canal structure was observed in the posterior half and only two canal structures were found in the anterior and medial explants. No canal structures were seen in any ventral explants. The cochlear structures were observed in the ventral, anterior, posterior, lateral and medial halves of the otocysts. No cochlear structures were found in the dorsal explants. Utriculosaccular spaces and their two associated maculae were observed in the ventral, dorsal, lateral and medial halves. Only one macula was found in the anterior and posterior specimens. It appeared that the utricule and saccule develop from the middle portion of the otocyst. It is concluded that the eleventh gestation day mouse otocyst has become the mosaic for the development of inner ear sensory structures. (supported by grants NINDS(NS08365), Deafness Research Foundation and National Foundation-March of Dimes).

315 DEVELOPMENT OF THE RETINOHYPOTHALAMIC PROJECTION IN THE RAT. C.A. Mason*, N. Sparrow*, and D.W. Lincoln* (SPON: A.O.W. Stretton). Dept. Anatomy, Univ. Bristol, England.

The cobalt sulfide precipitation technique was used to observe the development of the retinohypothalamic projection in postnatal rats from day 1 to day 49. In freshly isolated rat brains, one optic nerve was cut, dried and surrounded by vaseline, and a drop of 130 mM $CoCl_2$ placed on the nerve stump. Preparations were kept for 7 hours at room temperature or in the refrigerator overnight. Tissues were immersed in 2% ammonium sulfide for 30 minutes, then fixed in 1% glutaraldehyde-1% paraformaldehyde for two days. Frozen 60μ sections were cut and stained with Timm's silver-sulfide method for intensification of heavy metals. At all ages, a small number of cobalt-filled fibers (visualized as black against a gold background) were present in the ventromedial portion of the posterior fifth of both the ipsi- and contralateral suprachiasmatic nuclei (SCN). Between day 1 and day 7, a second projection was seen to arise from the optic chiasm traversing through the lateral borders of the SCN to their dorsal edge. This lateral projection (LP) consisted of very fine collaterals of the main optic axons which branched profusely once they had left the optic chiasm and frequently possessed triangular 'end-feet'. The density of the LP increased as more branching occurred and reached a maximum at day 17, then diminished both in fiber concentration and dorsal extent, to be nearly absent by day 35 and nonexistent by day 49. The lifespan of the LP corresponds with the establishment of the neuroendocrine rhythms controlled by ambient illumination. (Supported by the Nuffield Foundation and the Science Research Council).

316 DIFFERENT NEURONAL GENERATION PATTERNS MAY PRODUCE ALTERED CONNECTIVITY PATTERNS IN REGIO INFERIOR OF THE HIPPOCAMPUS. Dee Ann Matthews*, James E. Vaughn, Robert P. Barber* and Richard E. Wimer. Div. of Neurosciences, City of Hope Natl. Med. Ctr., CA. 91010.

Genetically-associated differences occur in the distribution of mossy fiber synapses upon pyramidal cell dendrites in the hippocampus of adult mice. We have suggested that this variability could be explained by different patterns of pyramidal neuron generation. Therefore, the generation pattern of hippocampal pyramids was examined by radioautography in mice of two inbred strains that display extremely different distributions of mossy fibers. The mice were exposed to a single pulse of ^{3}H-thymidine on one day during the developmental period consisting of embryonic days 11-19. The typical "inside-out" pattern of pyramidal layer development was found in the hippocampus of SM/J mice where the mossy fibers are divided into the usual supra- and infrapyramidal bundles. However, a totally different pattern of pyramidal layer development was found in BALB/cJ mice, and these mice virtually lack infra-pyramidal mossy fibers. BALB/cJ pyramidal neurons displayed an "outside-in" pattern of generation that was specific to that portion of Regio Inferior which lacks infrapyramidal mossy fibers. The typical "inside-out" pattern of pyramidal layer formation was found in the remaining portion of Regio Inferior and all of Regio Superior. The precise localization of the reversed generation pattern to the portion of BALB/cJ Regio Inferior which exhibits a unique distribution of mossy fibers strongly suggests that this reversal plays a major role in altering the mossy fiber connectivity pattern. (Supported by U.S.P.H.S. grants NS10284 and 1 F32 NS05310-01).

317 NEUROGENESIS IN THE DIENCEPHALON OF THE RAT: ANALYSIS OF AND OBSERVATIONS ON VARIOUS NEUROGENETIC GRADIENTS. James P. McAllister* (SPON: S. Nakajima). Dept. Biological Sciences, Purdue Univ., W. Lafayette, IN 47907.

Using thymidine-H^3 autoradiography, neurogenesis was examined in close serial sections throughout the entire diencephalon of the rat, and the results were evaluated with an emphasis on the nature of various proliferative gradients. In general, intensely-labelled neurons indicative of final mitotic activity were observed during days 13-19 of gestation in the epithalamus, days 13-18 in the dorsal thalamus, days 13-15 in the subthalamus, and days 13-19 in the hypothalamus.

From a detailed analysis of these findings, the following conclusions were drawn: (1)No marked tendency for the formation of large neurons prior to smaller ones appeared to exist within the diencephalon, although the magnocellular hypothalamic nuclei did arise relatively early in development. Thus, nuclear structures are contrasted from laminar structures of the brain by the lack of a neuron-size proliferative gradient. (2)A comparison of directional neurogenetic gradients within the diencephalon indicated that only a lateromedial pattern was common to all subdivisions, although such a gradient was not clear-cut in the hypothalamus. (3)A caudorostral proliferative gradient was present in thalamic regions, but entirely lacking in the hypothalamus. (4)A ventrodorsal gradient occurred within sub- and dorsal-thalamic regions, exclusive of the epithalamus, while neurogenesis spread dorsoventrally through the hypothalamus. (5) Sulcus limitans seemed to provide a common starting point for two neurogenetic gradients, one progressing ventrally through the hypothalamus and the other moving dorsally through all but the habenular nuclei of the thalamus. (This research was conducted in the laboratory of Dr. G.D. Das and supported by NIH research grant No. NS-08817-05.)

318 EFFECT OF AN ELECTRICALLY INDUCED SEIZURE ON BRAIN TYROSINE AND TRYPTOPHAN HYDROXYLATION IN VIVO IN YOUNG AND OLD RATS. M. Colleen McNamara*and A.T. Miller, Jr. Dept. Physiol., Sch. Med., University of North Carolina, Chapel Hill, NC 27514.

Decline in tolerance to stress is a prominent feature of old age, and one of the causes is probably a progressive reduction in the efficiency of homeostatic responses controlled by the brain. Since catecholamines are mobilized during stress, a decline in the ability of the brain to synthesize these compounds might be a factor in the reduction in stress tolerance, as well as an index of brain aging. To test this hypothesis, a single electroconvulsive seizure (ECS) (serving as an acute stressor) was induced in 40 young adult (9mo) and 40 old (24 mo) male rats. The effect on brain catecholamine synthesis was measured, at intervals following ECS, by determining the accumulation of dihydroxyphenylalanine (DOPA) and 5 hydroxytryptophan (5 HTP) in rats pretreated with a central decarboxylase inhibitor (NSD 1015). This accumulation permitted an estimation of the in vivo rates of hydroxylation of tyrosine and tryptophan. ECS induced an increase in brain DOPA concentration (maximal at 60 min after ECS) in both young and old rats, but the increase was much greater in the young animals. Brain 5 HTP was unchanged in both young and old rats. Measurements of the concentrations of brain tyrosine and tryptophan ruled out precursor availability as a factor in the response to ECS. It is concluded that reduced capacity of the brain to synthesize dopamine may be important in the age-related decline in stress tolerance.

319 ONTONGENY OF EMOTIONAL RESPONSIVENESS IN LAMBS: EFFECT OF MATERNAL DEPRIVATION ON OPEN-FIELD BEHAVIOR AND CIRCULATING CORTICOSTEROIDS. Gary P. Moberg and Valeria A. Wood*. Dept. of Animal Science, Univ. of California, Davis, CA 95616.

To study the interrelationship of the nervous and endocrine systems response to postnatal stress, lambs were raised either in social isolation (ISOLS), peer groups (PEERS), or with ewes in groups (CONTS). At 14 days of age the behavioral and adrenal cortical responses to brief isolation stress were studied. Lambs were observed in an open-field for an initial 5 min. period followed by a second 5 min. period during which a toy horse was present. Blood samples were taken prior to the testing, immediately following, and 45 min. after the test. The amount of plasma cortisol in each sample was later determined. In the open-field test both ISOLS and PEERS had a significantly longer latency to first movement than did the CONTS. The amount of vocalization, lines crossed, and the duration of movement of the ISOLS were significantly less than that displayed by the CONTS. However, for each of these behaviors the PEERS showed an intermediate response between the other two groups. When the horse was placed in the arena the ISOLS still had significantly less vocalization than the CONTS and took longer to make first contact with the horse than did the lambs in the other two groups. The PEERS spent significantly greater time in the vicinity of the horse than did the ISOLS or CONTS. No difference was observed between groups in the non-stressed levels of cortisol or in the adrenal response to the stress. These data indicate that on exposure to an open-field test, lambs raised in social isolation were behaviorally more withdrawn than normal animals while lambs raised in peer groups had behavioral responses similar to the normal animals. Regardless of the behavioral response, the adrenal response was maximal to the stress.

320 CELL GENESIS AND PATTERNS OF CELL MIGRATION IN THE ALBINO RAT SUPERIOR COLLICULUS: A ^{3}H-THYMIDINE STUDY. Michael J. Mustari* (SPON: R. D. Lund) Dept. Biological Structure, Univ. Wash. Sch. Med., Seattle, Wash. 98195

The pattern of cell generation in the albino rat superior colliculus has been studied in adult and fetal material. The period of generation is from embryonic day 13-18 with a rostral-caudal and lateral-medial gradient. Heavily labeled cells are found in all laminae of the superior colliculus on E14-17, in all layers except the stratum griseum superficiale on E13, and occasional heavily labeled cells are found in stratum griseum superficiale and stratum opticum on E18. Statistically there is a tendency for the mean of the distribution of heavily labeled cells to get more superficial from E13-18, but the results argue for a nonlaminar pattern of genesis in the superior colliculus. The pattern is of an overall inside-out order and this differs somewhat with the pattern seen in the chick optic tectum (La Vail and Cowan, 1971, Brain Res. 28: 421). For the most part, cells of almost all sizes are being produced over the entire proliferative period; however, the large multipolar cells of deeper layers of the superior colliculus are produced only on E13 and the smallest cells (marginal cells) of the superior colliculus are produced on E15-16. The above pattern is consistent with the concept of simultaneous production of several cell types from the ventricular epithelium on any given day. Study of short term material shows that the majority of cells destined for the stratum griseum superficiale will migrate through the invading retinotectal fibers. Patterns of migration as deduced from ^{3}H-thymidine fetal material will be discussed. (Supported by USPHS Grants EY-00596 and GM-00136 from NIH.)

321 EFFECT OF DECENTRALIZATION OF THE SUPERIOR CERVICAL GANGLION ON THE DEVELOPMENT OF THE ADRENERGIC INNERVATION OF THE RAT IRIS. C. Mytilineou and I.B. Black. Dept. Neurol., Mt. Sinai Sch. Med., New York, N.Y. 10029 and Dept. Neurol., Cornell Univ. Med. Centr., New York, N.Y. 10021.

Transection of the preganglionic fibers to the superior cervical ganglion in 2-3 day old rats prevents the normal ontogenetic development of tyrosine hydroxylase (T-OH) activity in the iris and diminishes the number of adrenergic nerve fibers and the degree of their ramification (Black & Mytilineou, Brain Res., 101:503, 1976). We now report that decentralization results in additional changes in the adrenergic neurons. Catecholamine histofluorescence demonstrated that whereas the normal iris contained nerve fibers running in pairs, the decentralized iris contained fibers that were single stranded with large varicosities and very thin intervaricose segments. The uptake of ^{3}H-norepinephrine (^{3}H-NE) by normal and decentralized irides was studied in untreated and reserpine pretreated rats. When the vesicular storage of the nerves was abolished by reserpine, the cytoplasmic uptake of ^{3}H-NE by the decentralized irides was 53% of control. However, in untreated animals with intact vesicular storage, the ^{3}H-NE uptake by the decentralized irides was 76% of control. This difference was statistically significant ($p < 0.005$) and indicated a proportionately greater vesicular storage by the neurons of the decentralized iris. Retention of previously taken-up ^{3}H-NE was also higher in the decentralized irides. Although T-OH activity in the decentralized iris was 40% of the control, there was no difference in the dopamine B-hydroxylase (DBH) activity. Since DBH activity is associated primarily with the storage vesicles, the latter results tend to confirm a disproportionate increase in either the size or number of ^{3}H-NE storage vesicles in the decentralized iris.

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322 DEGENERATIVE STAINING OF AGED RAT BRAINS. Jennings N. Naranjo* and Ernest G. Greene. (SPON: David F. Lindsley). University of Southern California, Los Angeles, CA 90007.

The effect of age in producing degeneration in rat brain was studied using a variation of the Fink-Heimer, reduced silver technique. Tanaka & Chen have reported that this method stains for anterograde and for retrograde degenerative changes, with the "dust" produced by retrograde degeneration being visible for up to two months after the brain has sustained damage. In aged brains we have found signs of extensive degeneration in all areas of the brain, being more conspicuous in tracts than in cellular areas. Young animals show negligible amounts of the silver dust, but a moderate amount of degeneration-product is seen in middle-aged animals. The most extensive damage is found in the optic nerve and fornix of old animals, but a substantial amount is present in corpus callosum, internal capsule, anterior commissure, and most other fiber tracts. In hippocampus, the amount of degeneration-product in the lacunosum-molecular layer is greater than that found in radiatum, providing support for the suggestion that the stain will prove effective in showing differential atrophy of brain structures as a function of age.

323 MODELLING OF NERVOUS SYSTEM FUNCTION AS AN INTERACTIVE SYSTEM. M. N. Czer
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The traditional procedures for the clinical assessment of brain function both reflect as well as influence a continuation of thinking about the nervous system as a reflex "sensory motor machine". A deliberate attempt was made to measure function in an objective fashion. Such an approach has served neurology to identify the presence of disease and its accurate localization. It has not however provided the opportunity to consider the long term treatment of the patient as a functional organism and the potential for compensation for the deficits in function. It is suggested that different assessment procedures are necessary for such an exploration.

A protocol will be described by which one samples not only the present function of the patient but the process of adaptation. The examiner provides various options both in terms of stimuli as well as feedback conditions. As an example, one may vary the channels of input, the time frame, or the salience of the stimulus. The examiner is thus sampling in a self-conscious way the conditions under which, for example, the aphasic may communicate his needs if that be the particular concern. The product of such an exploration is not merely a number of options that may be used in the patient's long term treatment by the examiner. Another parameter must be introduced into the examination in order to make it an adaptive process. The patient must experience what is being used by taking an increasingly active role in specifying them. An additional crucial objective is the degree of participation of the patient in the process. Feedback is provided to the patient in recognition of such contributions.

A model is being provided of an interactive system. It is the bilateral awareness that is a more accurate model of sampling of nervous system function in life as the organ for evolution of the organism.

324 NEUROTUBULAR DISRUPTION RETARDS BRAIN DEVELOPMENT IN PERINATAL RATS. Ted L. Petit. Dept. Psychol., Univ. Toronto, West Hill, Ontario, M1C 1A4, Canada.

Colcemid has been repeatedly shown to disrupt the function of neurotubules. The addition of colcemid to tissues in cell culture disrupts the movement of materials in the cytoplasm, and causes a corresponding breakdown of cell process elongation. This study examined the consequences of neurotubular disruption in the in vivo developing rat brain.

Beginning at three days of age, infant littermate Long Evans hooded rats received daily I.P. injections of either .7mg/kg colcemid or a corresponding amount of saline. All animals were sacrificed on Postnatal Day 21.

Colcemid treatment produced a 20% reduction in brain weight. There was a corresponding reduction in the thickness of the isocortex and corpus callosum. Rapid Golgi and Golgi Cox impregnated brains revealed a reduction in dendritic growth in treated animals. Some cells also responded with aberrant spine morphology.

These results indicate that the functional integrity of neurotubules is essential for normal neural development.

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325 DIFFERENTIAL LECTIN RECEPTOR CONTENT ON THE SURFACE OF NERVE GROWTH CONES OF DIFFERENT ORIGIN. Karl H. Pfenninger and Marie-France Maylié-Pfenninger, Sec. Cell Biology, Yale University, New Haven, CT. 06510.

Various types of neurons from rat embryos were grown as explant cultures for 3-7 days in vitro. With a series of well-defined lectin-ferritin conjugates (J. Cell Biol. 67: 333a, 1975), the membranes of the advancing growth cones (GC) were analyzed for superficially exposed carbohydrates. Live cultures were first incubated in glycoprotein-free 1% serum albumin medium and then cooled to 0-5° C or aldehyde-fixed (followed by quenching of free aldehyde). These cultures were exposed to the following ferritin-conjugated lectins (F-) at levels that were saturating for dispersed pancreatic cells: concanavalin A (F-ConA; spec. Glc/Man), wheat germ agglutinin (F-WGA; spec. GlcNAc), ricin I (F-R; spec. Gal), soybean agglutinin (F-SBA; spec. GalNAc), and Lotus tetragonolobus lectin (F-L; spec. Fuc). The labeled cultures were processed for thin section electron microscopy, and the density of membrane-bound ferritin particles was studied as an indicator of lectin receptor contents. In control experiments carried out in the presence of the respective hapten sugar, no binding was detectable. GC of superior cervical ganglion neurons exhibited high densities of receptors for F-WGA and F-R, but less for F-ConA and still fewer for F-SBA. GC from spinal cord bound F-ConA considerably, but less F-WGA and virtually no F-R, whereas those of dorsal root ganglia were heavily labeled with F-WGA but much less with F-R. Still different binding patterns were found on olfactory bulb and cerebellum GC. So far, no receptors for the fucose-specific Lotus lectin have been detected on any one of the GC tested. These findings indicate that nerve GC of different origin exhibit clearly distinct surface carbohydrate compositions. These differential membrane properties may be significant for the process of neuronal recognition.

326 GROWTH HORMONE LEVELS DURING SLEEP IN ELDERLY MALES. Patricia Prinz, Douglas Blenkarn*, Mark Linnoila* and Elliot Weitzman. Duke University Medical Center, Durham, N.C. and Dept. Neurology, Montefiore Hospital, Bronx, N.Y.

Previous studies of daytime plasma growth hormone (hGH) levels have generally failed to observe alterations in aged as compared with young subjects. However, because the largest physiological release of growth hormone generally occurs at night in association with slow wave sleep onset, one cannot conclude that growth hormone deficiencies are absent in the elderly on the basis of daytime studies alone. Eleven elderly men (aged 65-70 yrs.) and five young men (aged 22-26 yrs.) slept in the sleep laboratory for 3 consecutive nights with an indwelling venous cannula. Twenty minute blood samples taken during the last night of all-night sleep EEG recordings were radioimmuno-assayed for hGH levels. Both sleep patterns and hGH levels across the night were found to be altered in the aged. As compared to young adults, 8 out of 11 elderly men showed smaller or nonexistent peak hGH levels in association with sleep onset. Overall hGH levels as integrated across the entire night were also diminished in these 8 subjects and the overall hGH group mean was significantly lower for the elderly group. In addition, the well documented age-related alterations in sleep patterns (less REM and S4 and more wakefulness) were observed in all elderly subjects; however, the correlation between sleep variables and hGH levels was not strong in the elderly group, indicating that factors other than altered sleep patterns may influence nighttime hGH release in the aged.

327 NOREPINEPHRINE AND ACETYLCHOLINE SYNTHESIS BY INDIVIDUAL SYMPATHETIC NEURONS UNDER VARIOUS CULTURE CONDITIONS. Louis F. Reichardt*, Paul H. Patterson*, and Linda L.Y. Chun*(SPON: Margaret C. Nelson). Dept. of Neurobiol. Harvard Med. Sch. Boston, MA 02115

Dissociated sympathetic neurons from neonatal rats, when grown in the absence of other cell types, develop the ability to synthesize and accumulate radioactive norepinephrine (NE) and dopamine (DA), but little radioactive acetylcholine (ACh) from labelled precursors [ACh/(NE+DA)<.01]. In contrast, the population of neurons produces considerable ACh, but little NE or DA when grown in high concentrations of medium conditioned by previous incubation with any of a number of rat non-neuronal cell types [ACh/(NE+DA)>20]. Since the same number of neurons survive in each condition (0 or 60% conditioned medium) the results suggest that individual sympathetic neurons can develop the ability to produce ACh or NE depending on the environment in which they differentiate.

With intermediate concentrations of conditioned medium, the population produces both NE and ACh in significant amounts. To ask whether a single cell can simultaneously produce both ACh and NE, the individual neurons were grown in isolation under "cholinergic", "adrenergic", and intermediate conditions. These single neurons make detectable amounts of only one transmitter; that is, a particular neuron produces 5-50 fmol of one transmitter and less than 1 fmol of the other. When the culture conditions are changed, the percentage of cells committed to synthesis of each transmitter is altered. Thus regulatory circuits may restrict the number of transmitters that an individual neuron can make, perhaps forcing a cell to respond in a "flip-flop" fashion to the cholinergic signal. Despite being capable of ACh synthesis, these single cholinergic neurons retain the ability to take up and store NE, although at a much reduced rate. (Supp. by NIH and Amer. and MA Heart Assn's.)

328 ANTECEDENTS AND CONSEQUENCES OF ENDOCRINE STRESS RESPONSE TO WEANING IN COLONY-BORN RHESUS INFANTS. E. N. Sassenrath, G. P. Goo*, M. S. Golub*, A. G. Hendrickx* and J. L. Russell*. CA Primate Res Ctr, UC, Davis 95616

Post-weaning endocrine and behavioral observations of a 30 colony-born weanling rhesus macaques from a single birthing season were correlated with pre-weaning and post-weaning colony histories. Infants were raised with individually caged mothers or with mothers in outdoor group caging, weaned at 6 months, and placed in mixed sex peer groups of 2 to 4 cagemates. Plasma cortisol levels were determined prior to weaning (pre-WC) and at 1, 3, 7, 14, 28, 42 days after grouping. After 6 weeks in peer groups, cortisol elevations after 1 hour isolation in a novel environment were also determined. Maximum post-weaning cortisol blood levels (at 1 to 3 days) (MWC) ranged from 210 to 924 mg/ml. At 6 weeks, cortisol levels in the home cage (HCC) ranged from 97 to 277; cortisol levels after 1 hour isolation (IC) ranged from 189 to 437. MWC levels correlated negatively with the complexity of early experience with mother: a significant portion of high MWC responders did not have pre-weaning social experience or prior cage moves within the colony. Sex differences were not significant in the initial MWC levels, although they became apparent in the post-weaning peer environment. Among females, MWC correlated positively with pre-WC levels, with HCC at 6 weeks, and with IC. In males, there was no correlation between MWC and pre-WC, while MWC correlated negatively with HCC and IC. Among the dominant animals (#1 rank) in post-weaning peer groups, there was no significant correlation among pre-WC and MWC and post-weaning behavior. However, among the most subordinate animals in all post-weaning peer groups, pre-WC and WC correlated positively with the total frequencies of submissive behaviors. It is concluded that early experience with mother involving relevant environmental feedback can influence subsequent endocrine and behavioral responses to psychosocial stress. (Supported by USPHS Grant RR00169 and HD08658.)

329 BEHAVIORAL AND PHYSIOLOGICAL RESPONSES TO PYROGEN IN NEWBORN RABBITS. E. Satinoff, G. N. McEwen, Jr. * and B. A. Williams*. NASA-Ames Research Center, Moffett Field, Ca. 94035.

In previous work we found that 1-3 day old rabbit pups did not develop a fever when injected with a pyrogen and left for 2 hr in an ambient temperature (Ta) of 32°C. However, when they were placed in a thermally graded alley, pyrogen-injected pups selected gradient temperatures (Tg) significantly higher than controls and had higher rectal temperatures(Tr) at the end of the test. In the present work we explored this phenomenon further and also examined whether the pups could respond to the pyrogen by increasing their metabolic heat production. Fifty pups were injected with either Piromen (500 mg/kg) or the sterile saline vehicle,and placed at Ta's of 24, 30, or 35°C. Then they were placed in the thermal gradient and allowed to move to their preferred temperatures. Tr's were taken before and after this test. Six other pups were injected with pyrogen or saline on successive days and placed in metabolic chambers. Their O_2 consumption was measured for 2 hr at Ta 35°C.

Results indicate that pyrogen-injected pups held at Ta's of 30 or 35°C preferred significantly higher Tg's (mean=40.1°C) than controls (mean=37.7°C) and their Tr's increased a mean of 0.7°C vs 0.2°C for controls. Mean preferred Tg's were the same for all pups held at Ta 24°C, as was final Tr. There were no differences in the O_2 consumption of either group. Thus, newborn rabbits respond to a pyrogen behaviorally by selecting higher Tg's than controls, although they do not respond physiologically by increasing metabolic heat production.

330 CYCLIC NUCLEOTIDES AND BEHAVIOR IN AGED RATS. M. J. Schmidt, J. F. Thornberry and L. E. Hill. (SPON: J. A. Clemens). Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46206.

An attempt was made to correlate hormone-receptor interactions in the CNS with behavior as a function of aging.

Female Wistar rats 24 months of age were compared to 3 month-old rats in an open-field situation to quantitate motor activity and emotional behavior. The aged rats were less active than the young rats and showed significantly less emotional behavior: i.e. grooming, rearing, sniffing. These differences, which were present on the first day of testing, became more marked during two subsequent days of testing. When placed into isolation for 6-8 weeks neither the young nor old rats developed mouse-killing behavior.

Cyclic AMP and cyclic GMP accumulation in the presence and absence of norepinephrine was examined in vitro in slices of brain stem, cerebral cortex, and the limbic forebrain area from rats 3, 12 or 24 months of age. No age-related differences in resting levels of cyclic AMP or cyclic GMP were seen in any brain region. Norepinephrine elevated cyclic AMP levels in all areas and the magnitude of the norepinephrine-stimulation was the same at the 3 ages. However, in the cerebellum the elevation of cyclic nucleotides produced by kainic acid, a cyclic analogue of glutamic acid, was lower in the older rats.

Adenylate cyclase activity was determined in homogenates of the corpus striatum from rats 3 and 24 months of age. Basal, non-stimulated activity did not differ between the groups and dopamine was able to elevate cyclic AMP synthesis at both ages. However, there was a significant reduction (30%) in dopamine-stimulated cyclic AMP synthesis in the striata from the aged rats. This reduction was observed at all concentrations of dopamine tested.

331 ABNORMALITIES IN JIMPY MICE TRACEABLE TO MICROTUBULE DEFICIT IN ASTROGLIA. Robert P. Skoff* (SPON: B. Talamo). Division of Neuropathology, Johns Hopkins School of Medicine, Baltimore, Maryland 21205.

The mouse mutant, jimpy, is characterized by a paucity of CNS myelin. The myelin deficiency has been linked to an abnormality of oligodendroglial differentiation. The present study of the optic nerve demonstrates abnormalities in the number and migration of glia, and their organelle content. At 2 days postnatal, astroglia of normal animals have numerous microtubules but these organelles are virtually absent in glia of jimpy mice. At 2 dpn, jimpy astroglia have an average of 6 microtubules per sq. mm of cytoplasm while the heterozygous controls have more than 43. The branching of astrocytic processes is irregular at this time and this continues until their processes have surrounded most of the axons at 23 dpn. The migration of astrocytic precursor cells outward from the optic canal to the pia mater is reduced 50% at 2 dpn. Oligodendroglial migration also appears retarded, with most of them remaining near the center of the nerve. The number of astrocytes/cross section is normal but oligodendrocytes are reduced 57% at 10 dpn and 71% at 23 dpn.

The results of this study suggest that the myelin deficiency in jimpy is due to a combination of factors: reduction of oligodendroglia, abnormal migration of glia, irregular branching of astrocytic processes, and abnormal oligodendroglial differentiation. Because microtubules have been demonstrated to play a necessary role in cell proliferation and migration in many systems, the hypothesis is put forward that the paucity of microtubules in developing glia is the underlying cause for many of the abnormalities observed in jimpy. (Supported by NS 10580).

332 CRITICAL PERIODS FOR EFFECTS OF MATERNAL RESERPINE ADMINISTRATION ON SUBSEQUENT POSTNATAL GROWTH AND ON BRAIN AND ADRENAL TYROSINE HYDROXYLASE IN THE OFFSPRING. Theodore A. Slotkin and Jorge Bartolomé*. Dept. of Physiol. and Pharmacol., Duke Univ. Med. Ctr., Durham, N.C. 27710.

Pregnant rats were given reserpine (1 mg/kg, s.c.) once daily on days 6-8, 9-11, 12-14 or 15-17 of gestation. Pups exposed to drug on days 6-8 showed retardations in body weight gain through 17 days of postnatal age and in brain weight through 10 days; the effect was smaller in magnitude and of shorter duration in offspring exposed at the later time periods. Beginning at 10 days postnatally, rats exposed on days 12-14 of gestation showed increases in adrenal tyrosine hydroxylase (TH) which persisted into adulthood; this effect was accompanied by increased adrenal dopamine β-hydroxylase and accelerated turnover of catecholamine storage vesicles, suggesting the development of permanent changes in adrenomedullary activity. The adrenal enzyme elevations were not seen in rats exposed at earlier or later prenatal periods. Reserpine given on gestational days 12-14 or 15-17 produced deficits in whole brain TH in the offspring from postnatal days 25 to 45 and from 10 to 25, respectively. These data show that maternal reserpine treatment can cause delayed or permanent alterations in the offspring in postnatal growth and in catecholamine biosynthetic enzymes in both central and peripheral tissue. Critical prenatal periods exist for the different types of alterations, and the neurochemical changes do not appear to be related directly to effects on growth. (Supported by USPHS DA-00465 and DA-00006).

333 DEVELOPMENT OF THE NORADRENERGIC INNERVATION OF THE HIPPOCAMPUS. Rebekah L. Smith and Robert Y. Moore. Department of Neurosciences, School of Medicine, Univ. Calif. San Diego, La Jolla, Ca 92093

The hippocampus of the adult rat is characterized by its unique afferent stratification. The study of the development of these afferent patterns has been examined to determine potential mechanisms which may govern this synaptic specificity. The present analysis concerns the arrival and maturation of one of these afferent populations, the noradrenergic (NA) fibers arising from the locus coeruleus. In the adult, these fibers distribute predominantly to the hilus of the fascia dentata and the stratum lacunosum-moleculare of CA1. We have studied the development of this innervation by three methods. By radiochemical assay, the NA level on the day of birth is only 5 ng/g tissue, or 1% of the adult content. This increases 10-fold over the next 4 days to 50 ng/g. At this age NA fibers can be seen by the histofluorescent method to enter the regio superior through the cingulum pathway, but the fibers are scattered and the overall pattern of innervation is quite incomplete. At 24 days, the level of endogenous NA is only 250 ng/g, or approximately 50% of the adult. Somewhat by contrast, the capacity for hippocampal slices to accumulate ^{3}H-NA by high affinity uptake develops to more than 70% of the adult capacity as early as day 12. This suggests that the innervation may be more nearly complete at early stages than indicated either by the fluorescent histochemistry or endogenous amine determinations. (Supported by USPHS NS-12267)

334 LONG TERM EFFECTS OF VINBLASTINE INDUCED AXON DELETION IN CHICK EMBRYO TIBIAL NERVES. Betty G. Uzman, Gloria M. Villegas* and Jeanne M. Curnutt*. VA Hosp. and LSUMC-Shreveport, LA 71130 and I.V.I.C., Caracas, Venez.

Previously we reported in 60% of treated chick embryos (C.E.) the deletion of $\sim$ 1/2 the tibial nerve bundle neurites* 30-48 hrs. post injection into the chorio-allantoic sac (at 11 days incubation) of 0.5µg vinblastine sulfate (Velban, VBS)/g C.E. In typical experiments using $\geq$ 48 eggs so injected, 80-85% of embryos die before or after hatching (compared to a 30-40% prehatching loss of saline injected controls). The surviving 15-20% of VB treated C.E. (VB-C.E.) hatch independently or require assistance. At hatching and for the first week thereafter (0 to +7 days age) VB-C.E. exhibit varying aspects and levels of neurological abnormalities with constant toe flexion, hyperextension of legs, crouched stance, slow or unsteady gait and loss or weakness in righting ability as common features, the latter associated with prolonged, sustained lower limb tremors and nuchal rigidity. While average weights of VB-C.E. at hatching are low, rate of weight gain thereafter can parallel or exceed control rates providing a wider weight range on sacrifice at +42 days. A striking abnormality of tibial nerve fibers is limited to the proximal end (level of sciatic notch) where "monster" fibers with dense deposits in Schwann cells and/or axons are observed in varying numbers (3 - several hundred). These are not observed at mid-thigh level of the VB nerves, but at the same level in controls. Detailed ultrastructural observations of the nature and extent of lesions will be presented with quantitative analyses of fiber populations and their perikarya of origin at intervals up to +42 days when recovery of normal function is complete. Supported in part by a grant from the National Foundation-March of Dimes.

*Bundle neurites are distinguished from segregated, isolated and myelinating axons according to Webster, et al, Dev. Biol. 32: 401, 1973.

335 NEURONAL RESPONSE AUTORHYMICITY ASSOCIATED WITH NON-MECHANICAL FREQUENCY FILTERING IN THE ELECTROSENSE SYSTEM OF EIGENMANNIA VIRESCENS. Terry A. Viancour, Dept. Neurosci., Sch. Med., Univ. Calif. San Diego, Ca. 92093

Eigenmannia is a weakly electric gymnotid fish from S. America. The species produces a continuous, quasi-sinusoidal electric organ discharge (EOD). Each individual maintains a unique EOD frequency in the species range of 200 to 600 per second, and there is a concomitant selective frequency sensitivity in the reception-transduction process. In the EOD silenced animal the reception-transduction process has an inherent autorhythmicity. The compound action potential of the afferent nerve has an oscillatory waveform with a temporal structure related to individual EOD frequencies. Single units excited by receptor transduced pulsed electric fields respond with multiple action potentials which have stimulus polarity-dependent latency shifts, and the excitability/recovery cycle of these afferents can be oscillatory with relative hyperexcitable periods that are correlated with the unit's frequency tuning. To explain these data the reception-transduction process is hypothesized as resulting in a damped oscillatory receptor potential with a fundamental frequency at, or near the animal's individual EOD frequency. In the phylogenetically related mammalian auditory system a similar mechanism may exist to provide frequency filtering in addition to that provided by mechanically resonant cochlear structures.

Evoked Potentials and EEG

336 EVOKED EEG POTENTIALS AS AN INDEX OF CNS DYSFUNCTION IN MALNUTRITION.
A.B. Barnet, M.V. Sotillo*, I.P. Weiss, Child. Hosp. Nat. Med. Cntr., Washington, D.C. 20009; J. Cravioto*, M. Shkurovich*. Inst. Mexicana de la Asistencia a la Ninéx, Mexico City, Mex.

Sensory Evoked Potentials (EPs) were obtained from children who were being treated for severe malnutrition (marasmus) at the Hospital Infantil of IMAN in Mexico City. Auditory EPs to click stimuli recorded from vertex electrodes were examined in order to determine (1) their possible utility in quantifying the CNS dysfunction that may be a consequence of malnutrition, (2) the progress of the child during treatment, and (3) the possible residual CNS deficits following treatment. The 26 children studied were between one and 12 months of age at the time of hospital admission. The patients were discharged when their weight-age approached height-age, mean hospital stay being six months. Serial testing was performed during hospitalization, and when possible on post-discharge followup some months later. Same aged children from the IMAN day care center served as controls.

A numerical indicator of relative EP abnormality was developed based on a comparison of amplitudes and latencies of EPs obtained from the patients and day care controls with age-matched norms derived from a study of normal U.S. infants (Barnet et al. Electroenceph. Clin. Neurophysiol. 39:29-41, 1975). Scores ranged from 0 to 10, each score being the sum across seven EP components of the deviation of each component's value from the norm (1 or 2 sd). Differences among the mean scores thus obtained from EPs recorded at various stages during treatment of the patients, and from the controls led to the conclusions that: (1) EPs from marasmic children were significantly "worse" than those from controls; (2) EPs from marasmic children showed improvement during treatment; (3) EPs from patients discharged following rehabilitation remained worse than those of controls; and (4) Sex and height were significant cofactors in the degree of EP improvement shown by the malnourished children over their course of treatment.

337 COVARIANCE AND CORRELATION STUDIES OF RAW SENSORY EVOKED POTENTIALS. M. D. Berger* (SPON: M. S. Weiss). Naval Aerospace Medical Research Laboratory, New Orleans, LA 70189.

Study of covariance and correlation properties of raw sensory evoked potentials (EP) may yield better understanding of EP component structure, and EP-EEG relationships. Data were EP's due to transdermal stimulation of the median nerve, and recorded with bipolar, epidural electrodes from the cortex of adult Rhesus. System bandpass was .5 to 1700 Hz; inter-stimulus interval was 1 sec. A post-stimulus "reference time" (Tr) was chosen at a latency at which prominent EP activity was seen in averages. Using 1000 raw EP's, covariances were computed between Tr amplitude and amplitudes at times from 400 msec. pre-stimulus to 400 msec. post-stimulus at 2 msec. intervals. These were plotted similarly to an average EP, thus displaying a waveform of covariance properties relative to Tr. This plot corresponds to one row of a covariance matrix that might be used in factor analysis. Since EEG covariance properties appear in such a calculation along with EP information, as a control the same calculation was done with 2 msec. pre-stimulus as Tr. The pre-stimulus results using pre-stimulus Tr were subtracted from results using post-stimulus Tr with hope of isolating EP information. Similar calculations were done with product-moment correlations replacing covariances. The main purpose of this work was to determine whether and to what degree such calculations on raw data would yield reliable results.

Preliminary results follow. Except possibly with highest amplitude raw EP's (high stimulus levels), subtraction of pre-stimulus referred results from post-stimulus referred covariances does not isolate EP from EEG activity. Rather, EP and EEG related effects produce deflections of comparable amplitude in subtracted covariance curves. On the other hand, the EP shape, albeit modified, can often be seen reflected in the covariance curves, subtracted or not. This is the case when Tr is pre-stimulus or post-stimulus, but larger EP shapes are seen when Tr is post-stimulus. That this occurs at all with pre-stimulus Tr suggests that the commonly used model representing the measured EP as the linear sum of "ideal" EP and independent EEG is at best an approximation. It can probably be inferred from the data that the approximation is not adequate to use the model in factor analysis. Further work is needed on this. The fact that the EP shape is often larger when Tr is post-stimulus indicates first that EP amplitude is varying "in some measure independently of background EEG" as would be expected, and second that to a considerable degree, various parts of the EP measured tend to vary together. Similar results were seen in the correlation curves. Orderly latency dependence of EP variance was sometimes seen to produce shape differences between correlation and covariance curves.

Future work will concentrate on determination of the degree of interaction among various parts of the EP, and on attempts to improve the isolation of EP information by pre-selection of data according to pre-stimulus EEG and other criteria.

338 EEG SYNCHRONIZATION AND SLEEP - THE ROLE OF THE ANTERIOR RAPHE AND REGION OF THE AREA POSTREMA. Joseph D. Bronzino*°, Warren C. Stern, J. Patrick Leahy* and Peter J. Morgane, (SPON: John R. Bergen). Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545.

The anterior raphe system and region of the area postrema, including the nucleus tractus solitarius (NTS), have both been implicated in EEG synchronization and slow-wave sleep (SWS) mechanisms. The present studies were carried out to further investigate the involvement of the anterior raphe complex (especially the nucleus raphe dorsalis and nucleus centralis superior) and the area postrema-NTS region in synchronization and sleep. In these studies we examined the effects of electrical stimulation of these brainstem areas in the freely moving cat with the expectation that, if these subcortical regions are involved in the generation of EEG synchronization and/or sleep, then activation of these areas by low frequency stimulation would increase cortical synchronization and the occurrence of sleep in chronic animal preparations. Accordingly, sleep-waking profiles were obtained during chronic electrical stimulation of the region of the area postrema and anterior raphe nuclei in 8 cats. Results from 130 7-hr. stimulation-EEG recording sessions indicated that: (a) electrical stimulation of the region of the area postrema (0.5 or 10 Hz at 1 and 2 mA) significantly increased the occurrence of synchronization, SWS and REM sleep during the period of stimulation (compared to effects seen during stimulation of surrounding anatomical control sites) and; (b) the same stimulation parameters delivered to the nucleus raphe dorsalis and nucleus centralis superior and surrounding medial reticular areas did not alter the occurrence of any stage of sleep or induce EEG synchronization. We also examined the nature of the electrical activity obtained from the anterior raphe and area postrema-NTS area during the different states of vigilance utilizing power spectral analysis techniques. Since we had observed that the cortex exhibits distinct "spectral characteristics" depending on the state of vigilance (Bronzino et al. EEG Clin. Neurophysiol. 35: 187-191, 1973), we were interested in determining: (1) the vigilance related EEG spectral characteristics of the raphe and area postrema and; (2) if there was any correspondence between the power spectral characteristics obtained from the cortex, the area postrema-NTS region, and the anterior raphe nuclei. Using power spectral techniques we examined the frequency characteristics of the spontaneously generated EEG obtained from the anterior raphe, area postrema, and control sites adjacent to these regions, as well as the neocortex, during the various vigilance states in 10 chronically implanted, freely moving cats. In comparing these subcortical spectral characteristics with those obtained from cortical sites, it was found that during SWS the spectral curves from the region of the area postrema closely corresponded to the spectral curves obtained from the neocortex. The EEG of the anterior portion of the raphe region, although exhibiting some low-frequency components during all vigilance states, did not show significant increases in low-frequency activity as the animal shifted from waking to SWS whereas during this transition significant shifts to low-frequency activity were seen in the area postrema-NTS area. These studies further implicate the region of the area postrema in the generation of synchronized activity and slow-wave sleep. The role of the anterior raphe in synchronization and SWS is not supported by these results.

° Also at Trinity College, Hartford, Connecticut
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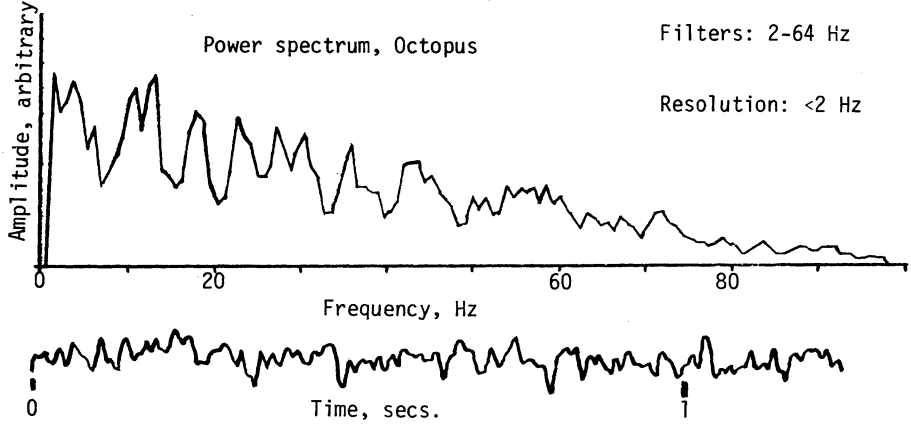
339 BRAIN WAVES OF OCTOPUS. Theodore H. Bullock and Thomas G. Uter, Neurobiol. Unit, Scripps Inst. Oceanography and Dept. Neurosciences, Sch. Med., University of California, San Diego, Ca. 92093

In several previous attempts we had failed to observe ongoing electrical activity from the exposed brain of anesthetized octopuses. Activity has now been recorded in each of eighteen animals by inserting electrodes through the cartilaginous cranium in the unanesthetized, semi-restrained state.

Eledone moschata and Octopus vulgaris of 150-200 gms were studied in Kotor, Yugoslavia, at 17-20°C (April-May); O. bimaculatus of 40-300 gms were studied in La Jolla, Calif., at 13-18°C (July-Jan.). Several types of semimicroelectrodes were used, especially short (10 mm) tungsten or steel needles, sharpened and plastic-insulated to a bare tip of ca. 10-15 μm length and with very light wire leading to the preamplifier. In larger animals the electrode was supported by the cartilage. In smaller specimens the electrode could be suspended by the fine, spirally wound wire, from a support positioned over the target before insertion; or it was inserted by a manipulator and then fixed with dental cement to an array of pins carefully placed in the cartilage beside and behind the brain. In each case the head was free to move, the body semirestrained by pinning, under temporary urethane, the arms either intact and similarly restrained, or amputated.

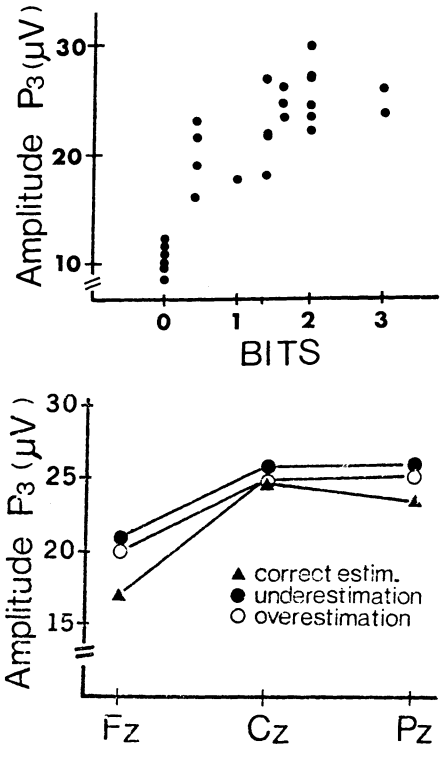
With a reference electrode in the sea water and one to four active semimicroelectrodes in the vertical lobe, 1-3 mm deep, no muscle or movement artifact is usually visible, although the animal is ventilating itself normally. There are long periods (tens of minutes) of electrical silence under our conditions. Activity may begin abruptly and spontaneously, without visible correlate in movement and last from a few seconds to several minutes. Prodding or light touch or flushing the mantle cavity may release some seconds of activity, if there has been a sufficient unstimulated period, but illumination does not. Control electrodes in cartilage are inactive; bipolar electrodes in the brain see activity; the activity probably comes from the brain. During a prolonged active period urethane reversibly flattened the record.

The character of the activity is highly variable but never similar to the usual spikey arthropod or gastropod brain activity. There may be runs of spikes (5-200 ms wide), usually 4-20 per sec. for a few sec. or periodically, every few sec.; or transient episodes of hashy small spikes as in a sciatic nerve; or longer epochs with mainly smooth sinusoidal waves of 20-50 Hz. Almost all samples are dominated by broad band (3=70+ Hz) activity without spikes or prominent single frequencies. The power spectrum resembles that of vertebrates more than that of crayfish, in falling from a maximum below 10 Hz to nearly control level above 70 Hz.



340 STIMULUS INFORMATION CONTENT AND THE LATE POSITIVE COMPONENT OF THE HUMAN EVOKED POTENTIAL. Kenneth B. Campbell* and Terence W. Picton. Faculties of Psychology and Medicine, University of Ottawa, Ottawa, Canada.

A parietocentral late positive component (P3) occurs with a peak latency of 300-400 ms in the evoked potential to a variety of signal stimuli. It has been proposed that this component might represent: 1) orientation to a relatively unexpected stimulus; 2) activity in a general-purpose information processing system; 3) activation of specific response systems appropriate to the processed information; 4) non-specific changes in state subsequent to the signal detection. In order to evaluate these possibilities evoked potentials were recorded to feedback stimuli providing information about performance on a time estimation task. Subjects pressed a button after an estimated one-second interval and one second later were given visual feedback (one of three light-emitting diodes) informing them about the accuracy of their performance. The information content of these stimuli was manipulated in two ways. Varying the window defining a "correct" time estimation altered the relative probabilities (and therefore the information content) of the confirming and disconfirming feedback stimuli without significantly changing task performance. The amount of relevant information in the feedback stimuli could also be varied by using quantitative (fast, correct, slow), qualitative (correct, incorrect) or meaningless (random) feedback schedules. This also allowed for differentiation between "surprisal" determined completely by stimulus probability irrespective of meaning, and "task-relevant information" determined by the meaningfulness of the feedback to the performance. The major determinant of the P3 component (peaking at 371, SD30 ms) was the amount of task-relevant information contained in the signal. The upper figure demonstrates this high correlation (r=0.89, $p<0.001$) between P3 amplitude and stimulus information. There was little correlation with stimulus surprisal. When the feedback stimuli were equally informative the P3 to disconfirming feedback was not significantly larger than to confirming feedback. However, scalp distribution studies showed (lower figure) that disconfirming feedback elicited a significantly more frontal P3 than confirming feedback ($p<0.02$). Thus the late positive component of the human evoked potential to feedback stimuli does not represent an orientation response because of its relative independence of stimulus surprisal; nor does it reflect a nonspecific change in state because of the definite differences between confirming and disconfirming feedback. Its amplitude is highly determined by the task-relevant information content of the feedback stimuli and its scalp distribution by the specific perceptual responses entailed by such feedback. It seems therefore to index both information processing and response activation.



341 EEG ASYMMETRY AND PERFORMANCE: VISUAL DETECTION OF WORDS AND PATTERNS IN THREE EXPERIMENTS. Charles S. Rebert. Dept. Psychobiology, Stanford Research Institute, Menlo Park, Ca. 94025

Whereas EEG alpha asymmetries have been shown to occur in different situations that presumably preferentially engage the left and right hemispheres, there has been no attention directed to the question of the actual significance of such differences in terms of their relationship to overt performance. Three experiments were, therefore, done to determine if interhemispheric fluctuations of alpha power occurring during task performance were related to reaction time (RT) in verbal and nonverbal target detection tasks.

In Experiment I Ss pressed a key as quickly as possible in response to target words or dot patterns generated by a Linc-8 computer and exposed for 50 msec, one every 1.5 sec, on a T.V. monitor. Verbal and nonverbal tasks were counterbalanced. Verbal targets were defined in terms of categories such as "animals," or "colors." Nonverbal stimuli were random dot patterns in a 3X3 matrix, targets specified by prememorization. In 7 of the 8 Ss alpha in the right hemisphere was enhanced in the nonverbal task, but when the R/L ratio was large, RT to words tended to be fast and RT to patterns slow.

In Experiment II the difference in the instantaneous mean square of alpha bursts in the left and right hemispheres was used to trigger the verbal or nonverbal stimuli. In some Ss, RT to words was fastest when the stimulus was triggered by a right hemisphere burst, whereas in others the opposite was true. However, it was generally true that the hemisphere trigger mode that resulted in fast RTs to words resulted in slow RTs to patterns, confirming the indication from experiment I that RTs to correctly detected targets depended on EEG asymmetry, but also indicating an unidentified factor related to individual differences.

In Experiment III verbal targets were 5-letter verbs, and nontargets were 5-letter nonverbs, while nonverbal targets were slanted lines with a dot above them and nontargets were slanted, vertical or horizontal lines with a dot below (or to the side) of them. These involved processing of spatial relationships. Responses were made with left or right hands and the sex of Ss was included as an experimental variable. Fewer target hits were made in the verbal task when the right hand was used and fewer were made in the nonverbal task when the left hand was used. Males made more hits than females in the verbal task. Reaction times were slower in the word task and faster in the pattern task when the right hand was used. These data suggested an "overloading"--performance being worst when a hemisphere was involved in both perceptual and motor functions. There was no overall effect of task on EEG asymmetry, but RT was a function of EEG asymmetry. RT was slower in the word task and faster in the pattern task when the R/L ratio was highest--contrary to expectation. Since right hand use appeared to engage primarily the left hemisphere (and vice versa) as indicated by less EEG habituation there with right hand use, there may be a common mechanism related to the effects of hand use and EEG asymmetry on performance.

Although differences in experimental procedures in these three experiments resulted in some apparent inconsistencies among them, they were consistent in indicating that overt performance depends on the state of EEG alpha asymmetry.

342 A SIMPLE METHOD FOR QUANTIFICATION OF SEIZURE ACTIVITY. S.I. Bellin* & H.L. Edmonds, Jr. Col. Pharmacy, Wash. St. U., Pullman, WA., 99163.

Characterization of convulsive states is commonly carried out by subjective observations of behavior and/or EEG recordings. Goldstein & Beck (Int. Rev. Neurobiol. 8:265, 1965.) quantified certain EEG alterations by use of an electronic integrator. This instrument converts EEG voltage changes into discrete pulses, the rate of which is proportional to the total energy content of the EEG. This technique, however, has not been applied to preconvulsive or convulsive phenomena. Eight adult male Sprague-Dawley rats were implanted with cortical screw electrodes and a device which permitted intracortical (i.c.) injections. Convulsions were elicited by i.c. or intraperitoneal (i.p.) injections of the epileptogen, picrotoxin. Integrated EEG activity and EEG recordings were made for 90 minutes. No drug was administered during the first 30 minutes. At the end of this epoch saline was injected i.c. or i.p. while at the end of the second epoch picrotoxin was similarly injected. Electrographic and behavioral concommitants of systemic and i.c. injections were compared to preinjection values. Saline injections did not result in significant alterations of EEG or behavioral activity. Systemic picrotoxin produced fewer afterdischarges (10±2 vs. 15±3) of shorter duration (28±21 vs. 46±12 sec.) than did i.c. administration. Clinical severity of convulsive episodes, measured on a scale of 0 to 4, was less marked in systemically injected subjects (2.1 vs. 2.8). These observations were reflected in the voltage integrated index. Systemic administration produced a 180% increase in total EEG energy content whereas i.c. administration enhanced total EEG energy 360%. Thus, the voltage integrated index may provide a simple technique for the precise quantification of seizure severity. (Supported in part by a grant from Washington State, Initiative 171 funds.)

343 EFFECTS OF HYPERBARIC OXYGENATION ON THE ELECTRICAL ACTIVITY OF THE BASAL GANGLIA. John G. Blackburn*, Robert W. Ogilvie*, and J. Douglas Balentine*. (Spon: S. Katz). Depts. Physiology, Anatomy and Pathology, Med. Univ. of S.C., Charleston, S.C. 29401.

The electroencephalographic activity and oxygen tension of the globus pallidus and corpus striatum (nucleus caudatus putamen) were recorded in anesthetized rats subjected to hyperbaric oxygenation (100% oxygen at 5 atm) for a one to three hour period. The oxygen tension and electrical activity were measured with a platinum-coated oxygen microelectrode. The slow wave electrical pattern of the globus pallidus was transformed into a desynchronized pattern of activity immediately following pressurization, followed by a gradual increase in voltage and frequency during the exposure period. These EEG changes were accompanied by a significant increase in local oxygen tension. The pattern of early desynchronization was absent in recordings from the corpus striatum which displayed an increase in voltage and frequency with time similar to the pattern of the globus pallidus. The oxygen tension of the corpus striatum was not changed over control values. The results of these experiments suggest a correlation between changes in local oxygen tension and electrical activity in the basal ganglia of rats exposed to hyperbaric oxygenation.

344 POWER DENSITY SPECTRAL ANALYSIS OF EEGS FROM DOGS ANESTHETIZED WITH HALOTHANE. C. A. Hanks* and E. L. Gasteiger. Dept. Physical Biology, N.Y. State College of Veterinary Med., Cornell Univ., Ithaca, N.Y. 14853

EEGs of mature and immature dogs anesthetized with halothane at various concentrations were subjected to power density spectral analysis to elucidate anesthetic induced factors that might modify the EEG. Dogs without premedication spontaneously breathed an accurately controlled mixture of halothane and oxygen. EEGs from transtemporal leads were taped for steady state, step changes, and sinusoidally varying concentrations of anesthetic. Power spectra were calculated for 32 sec epochs of EEG. Immature dogs were under ten weeks of age and in one case, spectra were obtained from the same animal at both 8 weeks and 8 months. In all cases, increases in halothane concentration up to 2.5% increased the power in frequencies below 5.0 Hz while not diminishing the power at higher frequencies. Indeed, in adult dogs there was also increased power in the 9.0-13.0 Hz band. In response to step changes from 1.0 to 2.5%, total power increased to a peak in minutes and then dropped to a plateau, with the puppies responding more quickly than adults. Nearly all the change occurred in the 1.5-3.0 Hz band. Tentative results for sinusoidal input (period = 7 min) show the total power to vary sinusoidally with different phase lags for various frequency bands. In puppies, the response was less regular and gave evidence for the existence of a narrow band of energy between 14.5 and 16.0 Hz. In our studies, power density spectral analysis has revealed subtle age and concentration dependent aspects of the EEG that are relevant to both clinical and basic research.

345 USE OF FACTOR AND MULTIVARIATE ANALYTIC PROCEDURES IN DETERMINING CLINICAL DISCRIMINATIONS OF COMPLEX EVOKED POTENTIAL MEASUREMENTS. Richard A. Roemer and Charles Shagass*. Eastern Pennsylvania Psychiatric Institute and Temple University Health Sciences Center, Philadelphia PA 19129.

Using factor analysis to identify clusters of related evoked potential measurements followed by multivariate discriminant methods appears to be a promising approach to resolving the large number of redundant measures obtained by electrophysiological studies. The data from a large study of somatosensory evoked potentials (SEP) gathered with a modified recovery function procedure were used to evaluate the utility of these multivariate procedures in discriminating clinical populations. Subjects were 56 nonpatients and 224 psychiatric inpatients.

Similar factor structures were determined for both the nonpatients (N=56) and the total group of subjects (N=280). Multivariate factorial discriminant analyses, using the clusters of variables identified by the factor analysis procedure, were then performed on a subset of the subjects. Analyses were performed with four clinical classifications of 20 subjects in each group matched for age and sex. SEP amplitude measures associated with single stimulus presentations resulted in significant clinical discriminations. Recovery functions of intensity-response slopes to a train of stimuli resulted in a significant diagnosis by sex interactions. A second recovery function to pairs of stimuli produced significant sex discriminations. These results confirmed the previous univariate reports, but with an unambiguous estimate of alpha.

Using factor analysis to isolate sets of variables, coupled with the use of discriminant analysis, appears to be a useful approach for dealing with the redundancy of electrophysiological measures.

346 A MODEL FOR THE ANALYSIS OF MULTIPATH SIGNAL PROPAGATION IN THE NERVOUS SYSTEM. Bernard Saltzberg, Neil R. Burch*, William D. Burton*. Information Analysis Section, Texas Research Institute of Mental Sciences, Houston, Texas, 77030.

Properties of the autocorrelation function are investigated as a basis for testing hypotheses concerning the presence of multipath signal propagation in the nervous system. The approach is based on the derivation of echo delay parameters which may be imbedded in a random process. The analysis is applied to complex EEG data to derive multipath time delay parameters which are virtually impossible to determine by visual inspection of the EEG time series. The model provides an interesting basis for studying deep brain pathology which may manifest itself electrophysiologically as an echo sequence of slowwave transient signals imbedded in background EEG activity. The relation of this model to cepstral analysis for investigating echo phenomena will be described.

347 WHITE-NOISE ANALYSIS OF THE VISUAL EVOKED RESPONSE. John Trimble. Dept. Ophthal., Univ. of Chicago, Chicago, IL. 60637.

The technique of white noise-analysis of non-linear systems was applied to the human visual evoked response (VER). VER's were obtained to light modulated with band-limited Gaussian noise. Computation of the first-order kernel revealed a waveform with marked similarities to early components of the transient VER. The amplitude spectrum of the first-order kernel compared favorably with the low-frequency regions of the VER frequency-response function obtained with sinusoidal stimuli.

Computation of the linear response from the first-order kernel indicated that non-linearities have an important role in determining the VER. However, there are no significant contributions to the VER from non-linearities higher than second order. Responses to double-pulse stimuli, predicted from the second-order kernel, were similar to those obtained experimentally.

The limitations of applying this analytical technique to the VER are discussed. In addition, the implications of the experimental data with respect to possible models of the VER are presented.

348 ATTENTIONAL SHIFTS ARE DETECTED IN REAL TIME IN SINGLE EPOCHS OF AUDITORY AND VISUAL EVOKED RESPONSES. Jacques J. Vidal, M.D. Buck, R.J. Hickman* and R.H. Olch*. Dept. of Comp. Sci., School of Eng. & Applied Sci., UCLA, Los Angeles, Calif. 90024

Last year the same authors reported the development of a remarkably successful procedure for the single epoch classification of visual evoked responses in man. The sensory stimuli used for the demonstration were unpatterned color flashes or flashed patterns with different retinal locations. In this approach the computer classifies each epoch in real-time and generates immediate feedback to the subject to report the success or failure of the identification process. The combination of real-time feedback with real-time artifact detection and an advanced application of stepwise discriminant analysis with multiple channels has led to an identification procedure that performed with near certainty in these early studies. More importantly, the decision rule created by the procedure was shown to constitute a descriptor for the response that is considerably more informative and specific than the customary averages.

The same approach has now been extended to the more challenging classification of responses that are triggered by identical sensory events but are modulated by shifts of expectation. Such components of central origin have been elusive in many earlier studies. It is shown that an extension of the same cybernetics approach leads to the improved detection of the active components and again to more specific information on their temporal and spatial distribution.

Epilepsy

349 UNILATERAL TRANSVERSE CORTICAL LESIONS RAISE AFTERDISCHARGE THRESHOLD BILATERALLY. John H. Ferguson, Gene H. Barnett, Howard J.Williams, and David R. Cornblath. Div. of Neurology, Case Western Reserve U. Sch. Med. Cleveland VA Hospital, Cleveland, OH. 44106.

Previous work(Ferguson, Neurology 22:412,1972) suggested that a transverse (coronal) cortical lesion (TCL) interrupting corticocortical connections between adjacent cortical segments would raise seizure threshold in these segments. Cats were chronically implanted with pial surface platinum ball electrodes, 2 stimulating and 2 recording, in two segments, posterior (P) and anterior (A), of each suprasylvian gyrus. In 2 control animals, no lesion was made; in 3, a single TCL was made between A and P segments unilaterally; and in 2, 2 TCLs were made A and P to one segment unilaterally. During each stimulation session, each of the 4 segments was tested by measuring peak current threshold for after discharge (ADT) to 2 msec duration positive-negative pulse pairs (50 Hz for 5 sec.). Sessions were purposely performed at irregular intervals, usually 1-2 per week, attempting to minimize any effect on ADT from the stimulation per se, either decreasing it as in "kindling" (Goddard and Douglas, Canad J Neurol Sci 2:385,1975) or raising it (Essig and Flanary Exp Neurol 9:31,1964). Results in tabular form are as follows:

				Mean ADT (ma ± std. error)			
Cat	Lesion	Sessions	Days	LA	LP	RA	RP
0111	0	8	42	.95±.09	.59±.04	.56±.04	.74±.09
0116	0	30	143	X	1.1±.13	.61±.06	.46±.04
508	LA/LP	16	124	3.4±.15	2.18±.07	5.47±.24	3.14±.33
0045	LA/LP	36	160	5.98±.5	1.68±.11	3.73±.17	6.73±.19
0049	LA/LP	8	98	3.7±1.23	1.06±.39	2.8±.55	1.01±.27
17	RA/RP/	21	83	3.11±.21	4.0±.33	5.89±.35	6.45±.37
124	/RA/RP	7	42	3.86±.78	3.43±.75	5.29±1.08	5.5±.91

Legend. LA=left anterior, LP=left posterior, RA=right anterior etc. Slash(/) = place of TCL. Sessions= no. of stimulation sessions. Days = no. of days over which sessions occurred. X= improper electrode connections. ma= milliamps.

Results indicate that in control cats, ADTs remained stable up to 143 days, mean ADT over all quadrants for all sessions in these 2 cats being 0.73±.02 ma. Varying session intervals did not seem to change ADT significantly. In contrast, ADTs in lesioned cats occasionally started near control values then rose over 1-3 weeks to levels 1.5 to 9 times higher bilaterally than that of controls. In 3 cats with a single TCL, ADTs though higher bilaterally than controls, were not necessarily higher on the lesioned side compared to the opposite side, but were always higher anterior to the TCL than posterior. Mean ADT for all stimulation sessions for each quadrant in these 3 cats was 4.99±.16 ma anterior to the TCL, 1.73±.04 ma posterior, with 4.07±.12 ma A, 5.01±.29 ma P on the opposite side. In 2 cats with 2 TCLs, A and P to one quadrant, ADTs were consistently higher in both quadrants of the lesioned side compared to the opposite side but not always highest in the segment between the TCLs. Similar to single TCL animals, ADTs were 4-8 times higher bilaterally than the mean ADT of control animals. These data support the original hypothesis in part, and in addition, suggest a bilateral elevation of ADT from unilateral single and double TCLs. It is tentatively concluded that this rise in ADT following the lesion is an effect related to the specific anatomical pathways interrupted both ipsi and contralaterally.

350 DEGENERATION FROM EXPERIMENTAL SEIZURES. A. Basil Harris, Dept. Neurol. Surg., Sch. Med., Univ. Wash., Seattle, Wash. 98195

Persisting degeneration of axons and boutons occurs in long-term (2-5 years) experimental epileptic monkeys in the cortical neuropil of the epileptic focus near intracortical alumina lesions. The present report details anatomical and electrical studies about alumina lesions of insufficient size to cause seizures (group #1), about inert beads inserted into the cortex (group #2), in cortex of epileptic foci brought under seizure control with anticonvulsant drugs (group #3), and in epileptic cortex where drugs were not given (group #4).

Small alumina lesions were placed in two animals in which no clinical or electrical seizures developed during one year following intracortical injections. In three other monkeys 1.5mm silastic beads were inserted into the sensorimotor cortex at sites identical to the injections. In four chronic epileptic monkeys (2-5 yr. seizure duration) the baseline seizure frequency was monitored by videotape and electrical changes were studied by EEGs for 1-3 mo. prior to commencing drug treatment with dilantin, phenobarbital and mysoline. Seizure control was maintained 9-14 months prior to final experiment. There were numerous monkeys in the uncontrolled seizure group. All animals were followed by frequent EEG tracings and the final experiment was performed under anesthesia with transdural electrocorticography. Brains were perfused with intravascular aldehydes and whole brain sections cut on a freezing microtome were studied with reduced silver methods of Nauta-Gygax and Fink-Heimer and Nissl stain.

Electrical abnormalities and clinical seizures failed to develop in the group with small alumina lesions and silastic bead intracortical insertions. In group three animals electrical changes were prominent prior to initiation of seizure control but became absent or markedly improved after clinical seizures were stopped. Transdural tracings likewise showed few abnormalities.

Reduced silver methods showed no degeneration in the cortex of group 1 and 2 animals. In group 3, seizure control animals, when the clinical and electrical abnormalities were stopped completely, no degeneration was seen. When control was good but incomplete, slight degeneration could be seen. In the group 4 untreated epileptic animals, degeneration was a prominent feature. All studies were done 2 years or more after injection lesions so that initial injury degeneration had been removed.

Chronic experimental epilepsy in monkeys is associated with chronic degeneration of axons and endings in foci. Degeneration is not apparent when epileptic activity, both clinical and electrical, is controlled with drugs for greater than six months. It is concluded that chronic recurring seizures result in degenerative changes in the brain.

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351 CEREBELLAR REGULATION OF SEIZURES: ANALYSIS OF POTENTIAL CONTROL MECHNISMS. R. N. JOHNSON, J. D. CHARLTON, S. R. QUINT, AND G. R. HANNA
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The cerebellum has been implicated as a potential regulator of seizure activity in both animal experiments and in recent, chronic cerebellar stimulations in humans. It is not known what the mechanisms of action are, or whether effective control of cortical seizures is mediated through the medial cerebellar system with its projections through the reticular formation, or via the lateral, cerebellothalamic projection system. In this study we have compared the effects of stimulation of paramedian lobule, lobulus simplex, red nucleus and mesencephalic reticular formation, and the effects of common anticonvulsant drugs on the excitability of the thalamocortical motor system of the cat.

Under chloralose anesthesia, stimuli were delivered to ventrolateral thalamus, with the evoked responses recorded from sensorimotor cortex. The average amplitude of each evoked response was calculated by an on-line computer and automatically stored as an ordered triplet with the corresponding conditions of stimulus amplitude and the time interval from the previous stimulus. A three-dimensional response profile of the system was then used to test the thalamocortical system in its control state and under the stimulation and drug conditions listed above.

Anticonvulsant drugs, such as diphenylhydantoin (DPH) produce a uniform reduction in the response profile. Stimulation at 100/sec of the contralateral paramedian lobule of the cerebellum produces both a nonuniform reduction in the amplitude of the response profile as well as alteration of its threshold. Stimulation of the red nucleus produces a nonuniform reduction in the amplitude of the response profile similar to paramedian cerebellar stimulation, but does not shift the response threshold. Stimulation of the mesencephalic reticular formation at the level of the red nucleus produces a general reduction in response amplitude similar to that for DPH. Stimulation of lobulus simplex contralateral to the thalamic stimulation results in complex changes in the response profile amplitude without changes in threshold. Significant residual effects remain after simplex stimulation ceases.

In a parallel set of experiments, we have studied the spontaneus alterations in the response profile which occur under conditions of penicillin induced cortical spike activity. These experiments demonstrate a process of dynamic regulation in which cortical spike activity begins, then the amplitude of the response profile is reduced and/or threshold levels are increased, followed by the abrupt cessation of cortical spike activity. Stimulation of both the paramedian lobule and lobulus simplex produceschanges in the response profile similar to those observed during spontaneus regulation of the response profile during conditions of penicillin induced cortical spike activity. These studies suggest that cerebellar stimulation controls seizure activity by the activation of an endogenous regulator, the cerebellum, and not simply as a consequence of a fortuitous shift in brain excitability levels.

352 REVERSIBLE CEREBRAL DE-AFFERENTATION AND SYMPATHETIC DE-EFFERENTATION BY MIDBRAIN COOLING IN CATS ANESTHETIZED WITH FLUROXENE AND ENFLURANE

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Gloor and co-workers have introduced the concept of "corticoreticular" epilepsy, in which generalized epileptiform EEG activity varies inversely with the tonic desynchronizing influence exerted by the midbrain reticular formation (MRF) on electrocortical (ECoG) activity. There is evidence that the cerebral irritation produced by certain general anesthetics is modulated by a similar corticoreticular mechanism,and we further tested this hypothesis by cooling the MRF of cats equilibrated at sub-epileptogenic concentrations of two stimulant anesthetics, fluroxene (2,2,2-trifluoroethyl vinyl ether) and enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether). Two stainless-steel cryoprobes, 1 mm in diameter, were inserted stereotaxically into the MRF bilaterally at coordinates of A: 2.0 to 3.0, L: 1.5 to 2.0, H: -3.5 to -4.0. After full behavioral and EEG recovery, the MRF was cooled to between 0° and 15° C. (depending on flow rate) by circulating cold ethanol through the probes under pressure. In all animals, this produced coma within 2-3 minutes which was associated with high-voltage slow waves in the ECoG. The coma and ECoG slowing fully reversed within 1 minute of rewarming the probes in these control coolings

After equilibration at alveolar concentrations of 9% fluroxene or 1.9% enflurane, respectively, bilateral MRF cooling precipitated spontaneous electrographic seizure activity. In the case of fluroxene, the ECoG seizure activity was nearly continuous -- interrupted only by brief interictal silences -- and continued for as long as MRF cooling was maintained. With enflurane, the seizure activity did not persist despite continued cooling although there was a persistent increase in the incidence and stimulus-sensitivity of enflurane-induced spike and wave activity; the number of spike-waves/minute and number of auditory stimuli required to entrain an electrographic seizure returned to their pre-cooling levels within 1 minute of cessation of flow through the cryoprobes. The effect of MRF cooling on epileptiform ECoG activity was less dramatic or absent at concentrations of the anesthetics other than those indicated above.

Fluroxene-induced seizure activity precipitated by midbrain cooling was not associated with the peripheral sympathetic effects -- tachycardia, hypertension and pupillary dilatation -- which accompanied the spontaneous seizures produced by higher alveolar concentrations of fluroxene (12-15%). When the midbrain was cooled after the spontaneous appearance of seizure activity at these higher concentrations, the associated tachycardia was reduced by 22% ± 5%. An even greater decrease in heart rate of 38% ± 3% was seen under these conditions in atropinized animals, suggesting that midbrain cooling reduced parasympathetic -- as well as sympathetic -- cardiac tone. However, the net sympatholytic effect of midbrain cooling in the non-atropinized animal suggests that descending parasympathetic fibers were less affected by cooling than the sympathetic pathways. Midbrain cooling in the unanesthetized animal also produced reductions in heart rate (by 19% ± 2%)in the presence of vagal blockade. Rewarming of the midbrain during fluroxene-induced seizure activity was followed by full return of all the peripheral sympathetic manifestations.

These findings indicate that reduction of reticular "activating" drive potentiates the cerebral-irritant effects of fluroxene and enflurane in the cat, just as it does for systemic penicillin [1]. Reduction of the sympathomimetic effects of fluroxene by midbrain cooling suggests a more central origin for some of these effects than has been proposed [2].

[1] Testa G, Gloor P: Electroenceph clin Neurophysiol 36:517-524, 1974.
[2] Skovsted P, Price HL: Anesthesiology 32:210-217, 1970.

353 EFFECTS OF CORPUS CALLOSUM SECTION ON THE PROPAGATION OF SEIZURE AFTERDISCHARGE TO CONTRALATERAL CORTEX AND THALAMUS. John A. Kusske and Jeffery L. Rush* Neurosurgery Section, VA Hospital, Long Beach and Div. of Neurological Surgery, UC Irvine

Corticocortical, corticothalamic and thalamocortical network dynamics were studied in cats with seizures induced by the injection of tungstic acid gel into the motor cortex of the pericruciate gyrus. The data represents the simultaneous recording of cortical and thalamic neuronal activity by tungsten microelectrodes in the region of the cortical focus, the ipsilateral ventral anterior thalamus and the homologous region of the contralateral gyrus and thalamus, during progressive stages in the maturation of the epileptogenic focus. The acquisition of data was from two groups of animals; the first with intact corpora callosum (CC) and the second with the CC sectioned. The development of seizures followed an orderly sequence in both groups of animals. Initially burst firing patterns and coinstantaneous paroxysmal slow waves were recorded at the focus. With the CC intact the slow waves were propagated both to ipsilateral thalamus and contralateral cortex with delays appropriate for transynaptic propagation. In 70% of the animals an alteration of firing patterns was not detected in the other recording sites outside the focus coincident with the propagated slow wave and in the remainder an inhibition of unit discharge was noted both in the ipsilateral thalamus and contralateral cortex along with the slow waves. With CC cut the slow wave was propagated only to ipsilateral thalamus. The onset of a generalized seizure was marked by the propagation of an initial slow to contralateral cortex and ipsilateral thalamus but the spread of seizure afterdischarge to contralateral cortex did not occur until the ipsilateral thalamus was activated. Subsequent to the appearance of afterdischarge in the contralateral cortex in the usual case, 5-7 seconds elapsed prior to involvement of the contralateral thalamus. Near the termination of each seizure there was an increase in the amplitude of the afterdischarge in both thalamic recording sites independent of the cortical focus or the contralateral cortex. In those instances where the CC was cut the spread of seizure afterdischarge involved activation of the ipsilateral thalamus followed by burst firing and self sustained afterdischarge in the contralateral thalamus some 6-8 seconds later. The involvement of the contralateral cortex was then marked by low amplitude afterdischarge and often by loss of unit firing, in marked contrast to the patterns of activity observed in animals with the CC intact, where all units appeared to be involved and where the amplitude of the afterdischarge was much greater. This study suggests that activation of the ipsilateral thalamus is necessary before transcallosal spread of seizure afterdischarge occurs. Activation of the contralateral cortex subsequent to CC section depends upon thalamocortical circuitry and the nature of the activity differs markedly from that recorded when the CC is intact. It is suggested that the intracortical neuronal circuits involved in the ictal activity subsequent to callosal section may vary considerably from those in use when the CC is intact.

354 PROPERTIES OF INTERICTAL EEG SPIKES IN THE LIMBIC SYSTEM OF PATIENTS WITH TEMPORAL LOBE EPILEPSY. Jeffrey P. Lieb, Barbara Leake*, Donald O. Walter , Antonio Siccardi*, and Paul H. Crandall*+. Reed Neurology Research Center, Sch. Med., UCLA, Los Angeles, CA. 90024.

Interictal EEG spikes, recorded from depth electrodes in the amygdala, hippocampal pes, and hippocampal gyrus in 4 patients with intractable temporal lobe epilepsy, have been analyzed with respect to their propagation and long-term temporal characteristics using computer oriented techniques. Automatic depth spike detection was done with a computer system which recognized EEG transients that were significantly sharper than background activity. Spike detections were then stored on digital tape for later plotting of rate vs time, point process analyses such as auto- and cross-correlation, and other statistical computations. These data were compared with the morphology of the spontaneous clinical and subclinical EEG seizure records of each patient. The results indicate that epileptogenic sites generate interictal spiking at a higher and less variable rate than non-epileptogenic sites. Propagation studies of interictal spikes were done with a computer system that uses morphologically defined spikes in a reference channel as triggers for the averaging of time-locked, concurrent activity in three other channels. Cross-correlation and cross-regression of these waveforms indicate that seemingly multichannel synchronous spiking in these patients is very often not synchronous; on the contrary, hierarchies among these waveforms can usually be established. For a given morphology, these hierarchies are stable across a period of several days. The phase relations observed for certain morphologies are the same as those observed for the beginning of the spontaneous clinical seizure record of the patient. Studies of interictal spike properties in depth appear to be potentially useful in predicting the side and site of seizure onset, as well as the course of seizure propagation, in patients with depth electrodes.

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355 CONTROL OF EPILEPTIC SEIZURES WITH LONG TERM SENSORIMOTOR RHYTHM (SMR) FEEDBACK TRAINING. Joel F. Lubar. Dept. of Psych., University of Tennessee, Knoxville, Tennessee 37916.

The purpose of this research program has been to assess the progress of patients with poorly controlled epileptic seizures who have had extensive sensorimotor rhythm (SMR) training and then have been gradually weaned from the treatment and followed after termination of their treatment. The methodology consisted of providing feedback for the production of 0.5 second epochs of EEG activity in the 12-15 Hz band of a predetermined microvolt level recorded from scalp electrodes placed bipolarly at C3-T3 and C4-T4. Detection of SMR was by a very precisely designed 14 pole elliptic filter and 40μV input clipper with greater than 80 db per octave rolloff. This filter attenuates harmonic frequencies by greater than 40 db and is as free from ringing as is possible up to the mathematical limit of current active filter design.

Feedback consisted of audio and visual information including lights and digital displays or slides. Patients were also provided visual feedback by one inhibit circuit which blocked the feedback for SMR when slow wave, epileptogenic activity, and spikes were present in the EEG. Additional inhibit circuits were employed to extract scalp EMG activity recorded from the active EEG electrodes.

The patients had frequent and poorly controlled generalized and partial seizures of the psychomotor, grand mal, myoclonic and akinetic types. They have been in training from one to three years. In the later phases of training they were gradually weaned from 3 treatments per week to one session or less per month. Some patients have now been entirely withdrawn from treatment. Throughout the study, spectral analyses were performed as well as clinical EEGs and blood analyses to determine if anticonvulsant drug levels remained constant. The reaults indicated that for most patients there was a gradual decrease in the intensity, the duration, and the frequency of their seizures. Significant correlations confirmed that there was a decrease in seizure activity over time. Some patients who initially had multiple daily seizures became seizure free for periods of up to several weeks. Those that showed the greatest seizure reduction also had the greatest degree of acquisition of activity in the 12-15 Hz bandpass and showed the greatest normalization of their EEG. Data from sleep studies of epileptics undergoing SMR training demonstrate increased stage 2 sleep spindles and decreased epileptogenic manifestations.

The most significant aspect of this study is that 12-15 Hz training of activity recorded over the sensorimotor area does seem to be highly beneficial in reducing the severity of epileptic seizures in the majority of our refractory epileptic sample. Furthermore, it is possible to maintain seizure control provided the patient is slowly weaned from training and provided they are encouraged to practice what they have learned during the training period.

356 MODIFICATION OF BARBITURATE WITHDRAWAL BY PHENYTOIN. Michiko Okamoto, Howard C. Rosenberg* and Norman R. Boisse.* Dept. Pharmacology, Cornell Univ. Med. Coll., New York, NY 10021.

Only limited information is available concerning the clinical efficacy of phenytoin in the treatment of the abstinence syndrome produced by chronic administration of sedative hypnotic drugs, the most often used examples being alcohol and the barbiturates. Those observations reported are controversial and often contradict each other, at least partly because of differences in the degree of physical dependence. We have recently established a reproducible animal model of barbiturate dependence. It was thought ideal to use this model system for studying the effectiveness of phenytoin in barbiturate withdrawal.

Severe physical dependency was produced in cats by twice daily intragastric administration of sodium pentobarbital in "maximally tolerable" anesthetic doses for 5 weeks (J. Pharmacol. Exp. Therap. 192, 555, 1975). The animals treated by this method displayed an intense abstinence syndrome that peaked 24-48 hours after the last pentobarbital dose.

The severity of withdrawal was assessed by counting the number of grand mal type convulsions and subjectively rating 20 additional motor, autonomic, and behavioral signs. EEG was continuously monitored through chronically implanted electrodes in some of these animals. Sodium phenytoin 10-40 mg/kg was given intravenously 24 hours after the last pentobarbital dose, near peak withdrawal intensity.

Peak serum phenytoin concentrations achieved and the elimination rate of phenytoin were determined gas chromatographically. It was found that even 10 mg/kg phenytoin was effective to suppress tonic convulsions but accentuated clonic convulsions, spastic rigidity, tremors, postural disturbances, and apprehensive behavior. Frequently, higher doses caused continuous clonic convulsions which was rarely seen in untreated barbiturate withdrawal. Phenytoin is an inappropriate drug for the treatment of barbiturate withdrawal. (Supported by NIDA research grant, DA00591).

357 ANATOMIC LOCALIZATION OF C^{14}-PENICILLIN IN EXPERIMENTAL FOCAL EPILEPSY. Timothy A. Pedley and Jeffrey L. Noebels . Dept.Neurology, Stanford Univ. Sch. Med., Stanford, CA 94305.

Topical application of penicillin is one of the most widely used methods to produce focal epilepsy. Though a significant amount of data has accumulated regarding electrophysiological events associated with this model of epileptogenesis, little is known about the penetration of penicillin into brain or its distribution in cortex at the time paroxysmal discharges appear. We applied gelfoam pledgets soaked in 80,000 U/cc C^{14}-penicillin to the suprasylvian gyri of adult cats. When well-developed interictal surface spikes appeared, the exposed gyrus was rapidly excised and immediately frozen in isopentane immersed in liquid nitrogen. Frozen sections parallel to the surface were cut at 100 μM intervals through the gray matter to a depth of 2.5 mm. Sample size was standardized by determining the protein content in each tissue sample using a fluorescamine reagent protein assay. Measured cpm could then be expressed in terms of the total amine content of each cortical slice. In other experiments, dry-mount autoradiography was performed on transverse sections of the gyrus. We found that 95% of the labelled drug is present in the uppermost cortical layers (laminae I-III) at the onset of interictal spike activity. Analysis of the concentration profiles obtained by radioassay showed that penetration of penicillin into brain occurs primarily by passive diffusion. Using standard diffusion equations, we calculated an apparent diffusion coefficient for penicillin in neocortex of 1.5 mm^2/hr. It is apparent that with a rapidly acting topical convulsant such as penicillin, the dimensions of the neuronal pool actually in contact with the drug will change over time and vary from experiment to experiment. Studies which attempt to characterize cellular pathophysiology within and around such experimentally produced epileptic foci must recognize and take into account the changing boundaries of the epileptic neuronal aggregate. (Supported by grants number NS 06477 and NS 12151 from the NINCDS, NIH)

358 BLOCKADE OF THE TONIC HINDLIMB EXTENSOR COMPONENT OF MAXIMAL ELECTROSHOCK SEIZURES IN THE MOUSE BY DRUGS ACTING ON MUSCLE AND MUSCLE SPINDLE SYSTEMS. Arthur Raines, Cinda J. Helke*, Michael J. Iadarola*, Lewis W. Britton* and Rebecca J. Anderson. Dept. of Pharmacol., Georgetown Univ., Schools of Med. and Dent., Washington, D.C. 20007.

Of the variety of experimental models used for demonstrating anticonvulsant activity, the maximum electroshock seizure (MES) test is perhaps the foremost. Blockade by drugs of the tonic hindlimb extension (THE) component of a MES in rodents is readily assessed quantally and large numbers of animals can be quickly studied. These facts have contributed to the wide use of the MES test in attempts to identify potential antiepileptic agents. The "anticonvulsant activity" of drugs in this context in preventing THE is thought to be due to a prevention of spread of the seizure discharge to brainstem centers. On the other hand, studies by Esplin and colleagues (Esplin and Laffan, Arch. Int. Pharmacodyn. 189: 1957; Esplin, Arch. Neurol. 1: 485, 1959; Esplin and Freston, J. Pharmacol. 68: 1960) have shown that peripheral factors are important in determining seizure patterns and suggested that feedback from tendon-organs of the flexors was important in leading to THE. The present experiments were performed to evaluate the influence of drugs which suppress motor function at a variety of levels on THE. Accordingly, C 28'882-Ba:2,4-di(diethylamine)-6-(phenylacetylhydrazino)-1,3,5-triazine, a compound described as a specific spindle suppressant (Bein and Fehr, Brit. J. Pharmacol. 19: 375, 1962) chlorpromazine hydrochloride, an agent which suppresses gamma motor neuron activity, gallamine triethiodide, a neuromuscular blocking drug, and dantrolene sodium, an agent which interferes with muscle contraction, were evaluated in the MES test after intraperitoneal injection in mice weighing 16-23 gm. The stimulus was a 50Ma, 60Hz stimulus of 200 msec duration applied with corneal electrodes. The first three agents abolished THE in the MES. The ED_{50}'s with 95% confidence limits for these agents were C-28'882-Ba, 0.54 (.47-.62) mg/kg; chlorpromazine 58.0 (45.5-74.0) mg/kg; gallamine 8.4 (7.1-10.0) mg/kg. Dantrolene did not abolish but significantly delayed the development of THE from 1.6 to 4 seconds after stimulus application. Likewise, MI-65-S:2-(3-dimethylaminopropyl)-1,3,3a,4,9,9a-hexahydro-4,9,-o-benzeno-2H-benz[f] isoindole dimethiodide, an agent which is also a muscle spindle depressant (Matsushita et al., J. Pharmacol. 147: 343, 1965) significantly delayed the development of THE.

None of the agents prevents the clonic seizures produced by 90 mg/kg of pentylenetetrazol subcutaneously. However, those agents which prevented THE in the MES also prevented THE in mice receiving a 200 mg/kg dose of pentylenetetrazol, a spectrum of activity resembling phenytoin.

The present studies support the contention that peripheral factors are a major determinant of seizure patterns, but focuses on the spindles of the extensors which are stretched by tonic contraction of the flexors. This leads to reflex excitation of the extensor motoneurons and THE. It appears that agents which undermine spindle responses by directly suppressing the spindles, decreasing their fusimotor support, or weakening the flexion can abolish THE. Interestingly, the spindle suppressant, C 28'882-Ba, appears to be the most potent antiextensor heretofore described and indicates that spindle suppression by phenytoin (Anderson and Raines, J. Pharmacol. 191: 290, 1974) may explain phenytoin's efficacy in blocking THE. These data emphasize that the interpretation of blockade of THE by drugs in the MES test requires considering many potential loci throughout the neuraxis. Supported by NIH Grant RO1NS10667.

359 THRESHOLD AND PROPAGATION CHARACTERISTICS OF SLOW WAVES IN BURST-FIRING CELLS. Howard Wachtel and Nels Anderson. Dept. of Physiology, Duke University, Durham, NC 27706.

From previous voltage clamp studies of burst firing neurons (BFN) in Aplysia we have derived a reciprocal current model for the slow waves underlying burst formation. This model, which is regenerative in nature (in keeping with the negative resistance characteristic (NRC) of these bursting neurons) not only simulates the clamped and unclamped behavior of the cell, but also predicts some unanticipated threshold and propagation possibilities. Threshold behavior is seen when the resting potential is set below (more negative than) the NRC range, and depolarizing pulses are delivered which lead to a single slow depolarizing wave (SDW). Using two (or more) coupled model cells, it is seen that the SDW can readily propagate from one cell to another, even in the absence of spikes. Furthermore, the model shows that coupled cells can produce propagated SDW's in concert even if each cell is incapable of producing slow waves on its own.

We have tested the predictions of the model directly on Aplysia BFN's (L3-L6) and also on uterine smooth muscle which exhibits similar activity. The threshold phenomenon is readily seen in the Aplysia BFN's and can be characterized by a strength-duration curve reminiscent of those associated with spike thresholds (but about 100 times slower). The possibility of intercellular propagation of the SDW was examined in the periodically contracting rat uterus using suction electrodes. Synchronized slow wave activity, not explainable by direct spike propagation, but consistent with the predictions of the SDW model, was seen.

The combined results from the model, the Aplysia BFN's and the smooth muscle experiments lead us to suggest that the SDW's are quite analogous to spikes in their threshold and propagation capabilities and can serve directly as a mode of regenerative communication between cells. In addition to its utility in explaining normally synchronous burst activity (as in smooth muscle) this notion may also be applicable to abnormal situations involving burst synchrony such as epileptiform activity.

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360 THE EFFECT OF DIPHENYLHYDANTOIN ON REGIONAL CNS TAURINE CONCENTRATIONS. Steven I. Baskin* and A. Jill Leibman* (SPON: B. Weiss) Dept. Pharmacol. Medical College of Pennsylvania, Philadelphia, Pa. 19129.

Taurine has been proposed to function in the central nervous system as an inhibitory neurotransmitter as well as an endogenous anti-epileptic agent. Diphenylhydantoin(DPH), a widely used anti-convulsant agentis known to induce seizures at high doses. The molecular mechanism of this action is unknown. Swiss-Webster mice were treated with a dose of DPH(100 mg/Kg) which was found to produce ataxia and convulsions. After one hour, animals were sacrificed and different brain regions(cerebellum, brainstem, cerebrum and midbrain) as well as heart, kidney and spleen were examined for their taurine (T) concentration. Taurine was separated by column chromatography, coupled to dinitroflourbenzene and analyzed using a method described by Baskin et al. (Taurine, Academic Press, 1976). In these studies involving DPH at a concentration which produced ataxia and convulsions, it was found to reduce the taurine concentration in areas, i.e. cerebellum and brain stem($p < .05$), thought to be responsible, in part, for the DPH-induced convulsions. It is proposed that the biochemical mechanism underlying seizure activity due to DPH is a reduction of the endogenous anti-epileptic compound , taurine. Funded in part, by the Pharmaceutical Manufacturer's Association.

361 ANTAGONISM OF THE ANTICONVULSANT EFFECTS OF DIPHENYLHYDATOIN AND ACETAZOLAMIDE BY 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT) AND 6-HYDROXYDOPAMINE (6-OHDA). R.A. Browning & R.L. Simonton*, Southern Illinois University School of Medicine, Carbondale, Illinois 62901

In order to further elucidate the specific role of serotonin (5-HT) and the catecholamines (CA) in seizure susceptibility, the anticonvulsant effects of diphenylhydantoin (DPH) and acetazolamide (AZM) were assessed in rats after the destruction of central serotonergic neurons by treatment with protriptyline (PTL) and intraventricular 5,7-DHT or after destruction of catecholamine neurons by intraventricular 6-OHDA. Anticonvulsant activity was measured as the ability of the drug to completely abolish hindleg extension in the maximal electroshock test. All seizure testing was performed at least 30 days after treatment with the neurotoxic compounds. Treatment with PTL (20 mg/kg, i.p.) plus 5,7-DHT (150 µg: i.vent.) was found to significantly reduce the anticonvulsant potency of DPH. A 12 mg/kg dose of DPH which protected 100% of control animals from hindleg extension failed to prevent tonic extension in any of the 5,7-DHT treated animals. Similar results were obtained when AZM was tested in 5,7-DHT treated animals, viz. a dose of AZM (12 mg/kg, i.v.) which abolished hindleg extension in 100% of the control rats was effective in only 16% of the treated group. Biochemical analyses revealed a 70-80% depletion in forebrain 5-HT with no reduction in norepinephrine (NE). The anticonvulsant activity of DPH was similarly reduced in rats treated with 6-OHDA (300 µg; i.vent.), but the effectiveness of AZM was only slightly decreased in these animals. For example, 12 mg/kg of AZM afforded protection in 83% of the controls and 33% of the CA deficient group. On biochemical analyses these rats exhibited depletions of 80-90% in forebrain NE and 60-70% in forebrain dopamine. These data suggest a nonspecific antagonism of anticonvulsant action in monoamine deficient rats.

362 THE DEVELOPMENT AND SUPPRESSION OF GENERALIZED SEIZURES TRIGGERED FROM NEOCORTICAL FOCI IN THE KINDLED RAT. W. McIntyre Burnham, Ronald J. Racine and Kenneth E. Livingston. Department of Pharmacology, University of Toronto, Toronto, Ont. (WMB and KEL) and Department of Psychology, McMaster University, Hamilton Ont.

Recently, we have been able to show that generalized seizures can be elicited from neocortical as well as limbic sites in the kindled rat. Generalization from neocortical sites tends to occur more slowly than from limbic sites, 30 or more stimulation days often being required to produce the first generalized convulsion. Neocortical generalization involves a progressive growth in afterdischarge similar to that previously described for limbic sites. The early stages of growth, however, are gradual rather than "incremental" in nature. Once generalization has appeared, the generalized component of seizures can easily be suppressed by massing stimulation or by "prefatiguing" the brain with a preliminary seizure triggered from a limbic site. Diazepam, which suppresses limbic seizures, also suppresses the generalized component of the cortical-generalized seizures. Procaine, which primarily suppresses cortical seizure activity, does not suppress cortical-generalized seizures, although it does appear to lessen the elements of the convulsion which are identifiably "cortical" in origin.

363 NEUROCHEMICAL CHANGES AND DRUG EFFECTS IN A MODEL OF EPILEPSY IN THE RAT. Daniel A. Callaghan* and Wayne S. Schwark. Dept. Physiol. Biochem. Pharmacol., N.Y.S. Coll. Veterinary Medicine, Cornell Univ., Ithaca,N.Y.14853

Repeated electrical stimulation of the amygdala (kindling) over a two week period resulted in a chronic convulsive disorder in adult male rats, as was reported by Goddard et al. (Expt. Neurol. 25: 295, 1969). Studies of the biogenic monoamines in different brain regions of kindled animals revealed that the level of hypothalamic norepinephrine was decreased to approximately 50% of that found in control rats. In contrast, norepinephrine content in the cerebral cortex, brain stem and basal ganglia was unaltered in kindled rats. The level of dopamine in the brain was unaffected by the chronic seizure disorder. The ability of acute administration of various antiepileptic drugs to abolish the seizures in kindled rats was examined. Although diphenylhydantoin sodium, phenobarbital sodium and ketamine hydrochloride were found to suppress the convulsions, this inhibition was accompanied by marked sedation. In contrast, diazepam produced suppression of seizure activity with little concomitant CNS depression. Additionally, chronic administration of ketamine hydrochloride during the kindling procedure prevented the development of seizure activity. The data indicate that this induced seizure disorder may be useful in studies on the pathogenesis and treatment of epileptic states in man. (Supported by USPHS, NIH Grants ES 00098 and NS 11137).

364 NEURONAL EVENTS IN THE AREA SURROUNDING PENICILLIN FOCI IN CAT NEOCORTEX. Kenneth R. Courtney, Jeffrey L. Noebels and David A. Prince. Dept. Neurology, Stanford Univ. Sch. Med., Stanford, CA 94305.

Neurons located in the area immediately surrounding penicillin foci in cat neocortex generate (1) a brief early excitation and (2) later and prolonged inhibition during surface interictal spikes (Prince and Wilder, Arch. Neurol., 16:194, 1967). These surface events are correlated with depolarizing shift generation and burst firing in cells located within the focus. Using intracellular recording techniques in barbiturate and non-barbiturate anesthetized cats, we now find that activities in neurons of the "surround" during interictal discharges are more complex than have been previously reported. In particular, a long-latency, long-duration depolarization is generally found within the IPSP of surround neurons. This late potential, which we presume is an EPSP, is usually "ragged" looking with several small spikelets appearing on it. The ragged EPSP is found in 75% of intracellular recordings from neurons recorded in penetrations 2 to 3 mm lateral to a small penicillin-soaked pledget (80,000 units/ml).

The ragged appearance of this late depolarization may signify the arrival of a burst of ectopically generated spikes of either antidromic or dendritic origin which are superimposed on the slow potential. Another possibility is that the intracellular microelectrode is recording presynaptic action potentials during the IPSP because of the large increase in conductance of the postsynaptic membrane. The long-latency, slow depolarization is clearly an effect of interictal discharge in neurons of the focus. Similar long-latency depolarizations occur in thalamic relay cells and neurons of contralateral homotopic cortex, although the circuitry and mechanisms involved in their generation are unknown. (Supported by NS12151 and NS06477 from the NINCDS, NIH.)

365 PHOTICALLY INDUCED SEIZURES IN ETHIOPIAN BABOONS, PAPIO CYNOCEPHALUS. Michael E. Corcoran, Donald P. Cain, and Juhn A. Wada, Div. Neuro. Sci., Univ. British Columbia, Vancouver, B.C., Canada.

Of a number of species of nonhuman primates tested, only Senegalese baboons (Papio papio) are known to display any significant degree of convulsive sensitivity to intermittent photic stimulation (IPS). This suggests that papio is genetically predisposed to certain forms of convulsive activity, and may be a unique primate model of epilepsy. In contrast to previous results, however, we have found that a high degree of photosensitivity is displayed by another species of baboons, suggesting that the exaggerated seizure susceptibility of papio may not be unique.

Six adolescent female Papio cynocephalus, captured in the highlands of Ethiopia, were purchased from a commercial dealer. After residing in our colony for 1 year, 4 of the baboons received implantation of chronic depth and cortical recording electrodes; EEG was obtained from the other 2 baboons with needle electrodes acutely inserted into the scalp. The baboons were adapted to primate boxes with moveable collars that allow free movement of the body in the vertical plane and rotation in the horizontal. After a period of gentling, the animals were exposed to IPS at a frequency of 25/sec. When tested at intervals of 3 - 12 days, 2 of the animals displayed seizures involving twitching of the head and face (C+2), 3 displayed generalized twitching of the body and limbs (C+3), and 1 was nonresponsive. In some cases driving was seen in the bulbar reticular formation, and epileptiform spikes were seen in the cortex. Thus, 5 of 6 cynocephalus displayed photosensitivity to a degree that has previously been reported only for papio. Others have presumably failed to observe photically induced seizures in cynocephalus because the animals were not sufficiently gentled before testing, or because photosensitivity varies with the age and geographical origin of the animals, as it does with papio.

366 PURKINJE CELL RESPONSES TO TRANSFOLIAL STIMULATION. George W. Dauth, Stephen Dell* and Sid Gilman. Dept. Neurol., Columbia Univ., NY 10032.

Purkinje cell (PC) activity is reduced during stimulation of the cerebellar cortex with parameter values similar to those used in humans with intractable epilepsy. (Dauth et al. Neurol. 26:362, 1976). In the present study PC activity was recorded extracellularly in cats anesthetized lightly with thiopental. Stimulating electrodes were two 1.8 mm diameter discs on 4 mm centers. At stimulus rates of 0.5 Hz, in most cases pulse durations above 250 μsec and currents above 600 μA were required to alter PC activity consistently. PC responses near the anode were similar to those near the cathode and alteration of PC activity was observed at distances of 0.5-6.0 mm from the electrodes. Units were not studied beyond 6 mm. PC responses to 0.5 Hz stimulation were complex; 17% were unresponsive, 30% responded with initial activation and 53% with initial suppression. Many showed complex subsequent alternations of activation and suppression. Elevation of pulse width or current values progressively enhanced the initial suppressive effects of stimulation. During stimulation at 10 Hz virtually every PC unit decreased in firing rate and many showed a rebound increase when stimulation was terminated. Thus, stimulation parameter values similar to those used in humans reduce PC activity over a wide area of the cerebellum. In the cat this area of suppression may exceed 14 X 18 mm. The intensity of suppression is related to the stimulus parameter values. The mechanism by which cerebellar stimulation alters epileptic activity is probably not PC inhibition of neurons in deep cerebellar and brain stem nuclei.

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367 POSTSYNAPTIC POTENTIALS IN PENICILLIN-INDUCED SPINAL MYOCLONUS IN CAT. J. Davenport*, P.C. Schwindt*, W.E. Crill. VA Hospital and Depts. of Physiology and Biophysics, and Medicine, School of Medicine, University of Washington, Seattle, WA, 98195.

Pencillin (PCN) applied topically to the cat spinal cord results in sudden depolarization shifts with high frequency spike bursts in many motor neurons. We used the well-defined mammalian segmental reflex pathways to determine whether these PCN-induced depolarizations are correlated with EPSP enhancement or IPSP decrement. Monosynaptic EPSP's, elicited in identified flexor and extensor motor neurons by electrical stimulation of Ia afferents and certain descending tracts, remained unchanged or slightly decremented after development of motor neuron bursting. Disynaptic IPSP's evoked from an antagonist muscle nerve and Renshaw IPSP's from ventral root stimulation were also unchanged by PCN. In contrast, polysynaptic PSP's from cutaneous nerve stimulation became considerably enhanced, prolonged, and often changed from inhibitory to excitatory as bursting developed. We conclude that PCN increases excitability in polysynaptic pathways, particularly those involving dorsal horn interneurons. This enhanced polysynaptic activity probably contributes to the generation of the motor neuron depolarization shifts. The temporal relation between development of motor neuron bursting and changes in presynaptic inhibition are under investigation. (Supported by VA Research Grant MRIS 1610, and NINCDS Grant NS05082)

368 FACILITATION OF AMYGDALOID KINDLING BY STRIA TERMINALIS LESIONS IN RATS. Jerome Engel, Jr. and Robert Katzman. Dept. Neurology, Albert Einstein Coll. Med., Bronx, N.Y. 10461.

Correlative electrophysiological and behavioral investigations of the development of amygdaloid kindling were carried out in two groups of male Sprague-Dawley rats. Four rats received RF lesions of the left stria terminalis at the time of electrode implantation and 4 served as controls. Kindling began on the 22nd postoperative day and consisted of daily bipolar stimulation of the left amygdala with 60 Hz sinusoidal 300 μa current. Generalized seizures appeared at 7 to 13 days in lesioned rats and 15 to 21 days in controls. This difference could be attributed to a foreshortening of the time required for the appearance of stage one kindling in lesioned rats (2 to 4 days) as opposed to control rats (10 to 13 days). Progression from stage one to stage five was not significantly different between the two groups. Lesioned rats also had longer afterdischarge durations from the beginning which appeared mature by the third or fourth day. Histofluorescent examination of the brains of 3 of the 4 lesioned rats revealed a decrease of fluorescent terminals in nucleus centralis of the amygdala on the left side. These results suggest that the increased local excitability observed during amygdaloid kindling in the lesioned rats may be due to destruction of catecholamine afferents reaching the amygdala via the stria terminalis. This is in agreement with pharmacological data demonstrating that reserpine and 6OHDA also facilitate kindling (Arnold et al., Exp. Neurol., 40:457, 1973; Corcoran et al., Exp. Neurol., 45:118, 1974). Experiments are in progress to correlate lesion induced alterations in catecholamine fluorescence with the pattern of kindling development. (Supported by USPHS Grants NS 09649 and NS 70209.)

369 QUALITATIVE CHANGES IN RETICULAR FORMATION UNIT RESPONSIVENESS INDUCED BY PENTYLENETETRAZOL. Carl L. Faingold, Division of Pharmacology, Department of Medical Sciences, School of Medicine, Southern Illinois University, Springfield, Illinois 62704.

Enhancement of sensory evoked responses is a consistent neurophysiological effect of a diverse group of drugs which induce seizures. The response enhancement has been previously shown to be maximal in non-primary reticular formation sites (Faingold, 1976). This study was undertaken to elucidate the neuronal mechanisms involved in the enhancement of evoked responses in the reticular formation. The responses of single units in reticular formation pathways to sensory stimuli were examined in unanesthetized paralyzed cats. The changes in response patterns of these neurons induced by the convulsant drug pentylenetetrazol (PTZ) were evaluated using post-stimulus time histograms. Units which were not responsive to sensory stimuli before PTZ became responsive to visual stimuli following PTZ administration as shown by the appearance of specific peaks in the histogram. Upon recovery from PTZ, the neurons returned to being non-responsive to the visual stimulus as shown by the lack of specific peaks in the histogram. This effect is modality specific to some degree, since the response pattern of the same units to auditory stimuli was essentially unchanged from the control situation in the units examined to date. The change in responsiveness was unrelated to changes in spontaneous activity. Spontaneous activity patterns at the time of the responsiveness change were either decreased or increased depending on the cell studied.

370 REGULATION OF ADENOSINE 3',5' MONOPHOSPHATE AND GUANOSINE 3',5' MONOPHOSPHATE LEVELS IN EPILEPTIC BRAIN. James A. Ferrendelli, Dorothy A. Kinscherf* and Kathryn L. Troyer.* Dept. of Pharmacology and Dept. of Neurology and Neurological Surgery, Washington University Medical School, St. Louis, MO. 63110.

Adenosine 3',5' monophosphate (cyclic AMP) and guanosine 3',5' monophosphate (cyclic GMP) were measured in anterior and posterior cerebral cortex, cerebellar vermis and hemispheres, striatum, thalamus and hippocampus of mice prior to and during seizures induced by pentylenetetrazol (100 mg/kg). In untreated (control) animals rapidly frozen in liquid N_2, cyclic AMP levels were 16 pmoles/mg prot in all regions of brain except cerebellum and striatum where the levels were 10 and 24 pmoles/mg prot, respectively. Cyclic GMP levels were 0.4 to 0.8 pmoles/mg prot in all regions except cerebellum where the level was 10 times higher. Levels of cyclic GMP in cerebellar hemispheres were consistently 25 to 50% lower than that in the vermis. Mild seizure activity (single myoclonic twitching or single clonic seizures) caused a 2-3 fold elevation of cyclic GMP levels in cerebral cortex, striatum and cerebellum. More severe activity (multiple myoclonic and clonic seizures or tonic convulsions) further increased cyclic GMP levels in these areas and also elevated its levels in thalamus and hippocampus. Both mild and severe seizure activity elevated cyclic AMP levels 2-3 fold in all regions except striatum where no change was observed. These data demonstrate that seizures do not have a uniform effect on either cyclic AMP or cyclic GMP metabolism throughout brain. This, in turn, suggests that the changes in cyclic nucleotide levels produced by seizures are related to select alterations of nervous function.

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371 EFFECTS OF EXPERIMENTAL EPILEPSY ON THE SEXUAL BEHAVIOR OF CATS. Frank P. Gullotta*, Dennis M. Feeney and Wendy Gilmore*, Depts. Psychol. & Physiol., Univ. New Mexico, Albuquerque, N.M. 87131.

The human clinical literature suggests that hyposexuality frequently appears in temporal lobe epilepsy but rarely occurs with other forms of epilepsy. This experiment systematically tests the hypothesis that an irritative focus in the temporal lobe results in hyposexuality. Baseline brain activity and sexual performance was recorded in male cats. Focal epilepsy was then induced by injection of aluminum hydroxide unilaterally into either basolateral amygdala (temporal lobe group) or anterior sigmoid gyrus (motor cortex group). Both groups gradually developed EEG and clinical signs of epilepsy.

Animals with an epileptic focus in the temporal lobe exhibited a dramatic decline in or a complete suppression of sexual behavior compared with baseline or control group performance. In contrast, over repeated sessions, motor cortex and normal cats slightly increased their sexual performance. Decreases in sexual activity in the temporal lobe group were correlated with the appearance of interictal spiking and at times preceded obvious clinical seizure activity. There was no relationship between epileptic EEG activity and sexual behavior in the motor cortex group. These data indicate that hyposexuality is a unique concommitant of temporal lobe epilepsy and that it is the result of abnormal and excessive neuronal activity in structures which normally have an inhibitory influence on sexual behavior.

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372 FASTIGIAL NUCLEUS STIMULATION IN GENERALIZED PENICILLIN EPILEPSY. John J. Hablitz and Gary Rea*. Dept. of Neurophysiology The Methodist Hospital and Baylor Col. of Med., Houston, Tex 77030.

Previous work from this laboratory has shown stimulation of the cerebellar cortex to be effective in suppressing paroxysmal discharges elicited in cats by parenteral administration of large quantities of penicillin. Since surface stimulation can theoretically cause increased or decreased output of the intracerebellar nuclei depending upon whether Purkinje cells are directly excited or suppressed via inhibitory networks, the mechanism of cerebellar suppression of epileptiform activity is not known. In the present study, surface and nuclear stimulation of topographically related areas of the cerebellum were compared for effectiveness in reducing abnormal discharges.

Stimulation of Larsell's lobules V & VI was effective in suppressing epileptiform discharges in chronic cats. Fastigial stimulation at high frequencies (100-200 Hz) also resulted in a significant reduction in the number and duration of paroxysmal events. Low frequency stimulation (10-20 Hz) did not significantly alter paroxysmal activity. Preliminary studies of dentate stimulation at high frequencies demonstrated a tendancy to reduce the number of seizures; low frequency stimulation did not affect the number of paroxysmal events but caused a slight but significant increase in duration. These findings suggest that the inhibitory effect of vermal and fastigial stimulation is mediated by increases in reticular outflow resulting from (1) direct activation of fastigial bulbar pathways by nuclear stimulation and (2) disinhibition of fastigial cells resulting from Purkinje-cell inhibition by surface stimulation.

373 THE ROLE OF SCAR FORMATION IN EPILEPTOGENESIS: PROPHYLACTIC EFFECT OF STEROIDS. Thomas J. Hoeppner* and Frank Morrell (SPON: W. H. Harrison). Dept. Neurology, Rush-Presbyterian-St. Luke's Med. Center, Chicago, Il. 60612.

It has been claimed, but never firmly established, that scar formation is an etiological agent in post-traumatic epilepsy. The following experiment examines the role of scar formation in the development of an epileptogenic focus.

Chronic epidural electrodes were implanted in twelve guinea pigs. A standard injection of metallic aluminum powder (an epileptogenic agent which produces a strong fibrotic reaction) was made into superficial cerebral cortex. To reduce scar formation half of the animals were given 0.16 mg of Meticortelone^{R} brand of prednisolone sodium succinate in 0.03 cc of 0.9% saline i.p. once per day starting on the day of surgery. The other half were given saline injections only. Three days per week electrographic recordings were obtained from each animal and the animals observed for seizure activity.

At the end of 4 months 6 of the 6 control animals (saline group) and only 2 of the 6 experimental animals (steroid group) showed epileptogenic spikes in the recordings. Moreover the degree of abnormality was greater for the saline injected animals.

Thus prednisolone was effective in controlling the development of an aluminum induced epileptogenic focus. The mechanism of this action is not yet clear, but converging experiments in scorbutic animals suggest that control of scar formation may be a critical factor.

374 EXCITATORY EFFECTS OF PENTELENETETRAZOL, TRIMETHADIONE, AND CHLORDIAZEPOXIDE ON SINGLE NEURONS OF THE BUCCAL GANGLION OF APLYSIA. N. R. Kreisman, M. F. Murphy* and W. M. King*. Dept. of Physiology, Tulane University, New Orleans, LA 70112

Pentelenetetrazol (PTZ) 5-50mM, chlordiazepoxide (CDZ) 0.1-0.5mM and trimethadione (TMO) 5-10mM were tested for their effects on identified interneurons and their follower cells in the isolated buccal ganglion. Each agent produced membrane depolarization accompanied frequently by bursts of spikes superimposed upon depolarizing shifts. With higher doses of TMO and CDZ, and occasionally with PTZ, this depolarization led to diminution of spike amplitude and complete blockade of spike generation within 30 min. TMO or CDZ applied 10 min. after PTZ produced transient potentiation of PTZ's excitatory action which was followed either by continued heightened activity or complete spike blockade. This potentiation was in marked contrast to antagonistic effects noted previously in abdominal ganglion neurons. All effects were reversible upon washing. Experiments were repeated in the presence of high Mg^{++} low Ca^{++} sea water to eliminate the contribution of chemical synaptic input to these responses. Although fast EPSP's and IPSP's were completely blocked, prolonged slow membrane changes remained, which we attribute to electrical coupling between buccal neurons. The excitatory and potentiating effects of PTZ, TMO, and CDZ persisted despite synaptic blockade indicating that the primary effect of these agents is postsynaptic in origin. (Supported by grants from NIH, NS-12419 and the Schleider Foundation of New Orleans).

375 EFFECTS OF FOOD DEPRIVATION ON AUDIOGENIC SEIZURE INTENSITY IN RATS. H. E. Laird II, P. L. Bates*, L. Chin and P. M. Dombrower*. Dept. of Pharmacology & Toxicology, The University of Arizona College of Pharmacy, Tucson, Arizona 85721.

In evaluating phenytoin against audiogenic seizure (AS) in rats, we observed that overnight food deprivation increased seizure intensity. Subsequently, the effects of brief (23 hr) and prolonged (4 days) food deprivation on AS intensity were investigated. Male audiogenic rats with a relatively stable AS intensity were used. Intensity of AS response was quantified by means of ranked scores (0 = no convulsion to 9 = maximal convulsion). Following a 23-hour fasting period, 5 of 16 rats showed an increase in AS intensity. The mean AS score after fasting was 2.8 ± 0.3 as compared to a pre-fasting AS score of 2.2 ± 0.2 ($p<0.05$). After 1 day of feeding, the mean AS score declined to 2.3 ± 0.3. Non-fasted rats ($n = 12$), tested at the same times showed no change in AS intensity. Rats subjected to 4 days of fasting ($n = 6$) showed a dramatic increase in AS intensity, from a pre-fasting AS score of 2.5 ± 0.6 to a post-fasting AS score of 6.2 ± 1.1 ($p<0.01$), whereas non-fasted rats tested at the same times showed a constant AS intensity. This enhanced AS intensity produced by prolonged fasting was still evident 12 days after the resumption of feeding (mean AS score = 6.0 ± 0.7). This study shows that food deprivation intensifies AS. The ramifications of this finding are obvious. For example, in the study of anticonvulsant drugs, promotion of gastrointestinal absorption of a drug by fasting should be weighed against the AS-enhancing effect of fasting. On the other hand, the AS-enhancing effect of fasting may provide us with a technique for producing severe AS for certain types of experimental convulsive disorder studies. (Supported in part by a grant from the Epilepsy Foundation of America).

376 SEIZURES OF AXIAL STRUCTURES: PRESUMPTIVE EVIDENCE OF BRAIN STEM ORIGIN. Morton Nathanson*, Allan Krumholz and David Biddle. Div. Neuro., LIJ-HMC Campus, Sch. Med., SUNY at Stony Brook, New Hyde Park, N.Y. 11040.

It has been postulated that some paroxysmal disorders of axial structures (face, tongue, palate, posterior pharynx, diaphragm, abdomen, etc.) actually may be seizures originating in the brain stem. Evidence from animal experimentation tends to support this assumption. We present two cases documented clinically, by motion pictures and electroencephalography, that not only prove that epileptiform seizures of axial structures do occur but add further support that the brain stem is their site of origin. Both patients were in coma with unequivocal brain stem signs when the seizures of axial structures occurred. In one case the electroencephalogram showed paroxysmal seizures during the attacks, followed by electrical silence corresponding to the interictal periods. In the other, each seizure was not only identical but had a rostral-caudal march of events terminating in gross diaphragmatic and abdominal muscle movements associated with expiratory grunts. The limbs were not involved. The structures and pattern of involvement, the episodic nature, the similarity of each attack, the interictal absence of motor and EEG activity along with the other signs of brain stem disorder make a compelling argument for these events to be classified as true seizures and implicate the brain stem as their site of origin.

377 PENICILLIN ALTERS EXCITABILITY OF MAMMALIAN PRESYNAPTIC TERMINALS IN VITRO. Jeffrey L. Noebels and David A. Prince. Dept. Neurology, Stanford Univ. Sch. Med., Stanford, CA 94305.

Pathophysiological changes in excitability at presynaptic nerve terminals can produce repetitive bursts of spikes which may propagate antidromically into the parent cell bodies. Such antidromic bursts occur in thalamo-cortical neurons during focal epileptiform discharges produced by the cortical application of penicillin or strychnine. These spike bursts arise independently of synaptic control mechanisms and can profoundly disturb normal patterns of neural signalling. We studied the mechanisms underlying this phenomenon by exposing isolated rat phrenic nerve-hemidiaphragm preparations to Na-penicillin and recording phrenic nerve action potentials across a sucrose gap. Within 2-3 minutes after exposure of the preparation to Tyrode solutions containing as little as 2.5 mM penicillin, single orthodromic stimuli to the nerve could elicit repetitive antidromic discharges. The bursts of action potentials disappeared following section of the nerve near the diaphragm, showing that they originated at or near the motor nerve terminals. Prolonged exposure of the nerve trunk to penicillin following isolation from the neuromuscular junction did not produce repetitive activity. In some preparations, penicillin initiated high frequency, asynchronous action potentials generated spontaneously at individual presynaptic terminals. Reduction of synaptic transmission by curare had no apparent effect on the terminal excitability changes caused by penicillin, suggesting that the latter do not necessarily depend on postsynaptic membrane depolarization. (Supported by NS12151 and NS06477 from the NINCDS, NIH.)

378 CYCLIC NUCLEOTIDES IN FOCAL PENICILLIN EPILEPSY. W. Raabe, S. Nicol*, R.J. Gumnit, N.D. Goldberg*. Depts. Neurol. Pharmacol. Univ. Minnesota, Minneapolis, MN 55455.

Epileptic foci were created in cat sensori-motor cortex by topical application of penicillin. Electrocorticograms were recorded from the focus in the left postcruciate gyrus, the homologous contralateral cortex, and the parieto-occipital cortex. Only the focus showed epileptic discharges; the activity recorded from the contralateral and parieto-occipital cortices was normal. The widely exposed cortex was frozen with liquid nitrogen during the various stages of epileptic activity, i.e. the period of inter-ictal spiking, the ictus and the post-ictal depression. Cortical tissue from the focus and the contralateral and parieto-occipital cortices was analysed for the concentrations of cAMP and cGMP.

Cyclic AMP levels in the focus were in general comparable to those in the remainder of the cortex, and did not change with the various phases of epileptic activity. Cyclic GMP levels in the focus were during all phases of epileptic activity significantly increased compared to the remainder of the cortex which contained 0.48-0.65 pmoles cGMP/mg protein. The highest cGMP-levels occurred during the ictus (6.73±0.65 pmol mg); during the phases of postictal depression and inter-ictal spiking cGMP was significantly lower than during the ictus (3.30±0.31 respectively 2.00±0.66 pmol/mg). In the epileptic focus produced by penicillin cGMP is markedly increased and undergoes changes with the various phases of epileptic activity. It appears that the increase of cGMP in the focus is related to the epileptogenic action of penicillin on the cortex.

379 AUDIOGENIC SEIZURES IN DBA/2J MICE: INDUCTION BY 15 to 30 kHz MONOTONIC STIMULATION. Robert A Schreiber , Brain Res. Inst., Univ. Tn. Ctr. for the Health Sciences, Memphis, TN 38163.

Some animals, when subjected to a sufficient acoustic stimulus, will enter into a full clonic-tonic convulsion, which may well terminate in the death of the animal. This phenomenon has been described in considerable detail for DBA mice in particular, which are considered by some investigators to be a natural model for the study of epilepsy and organic hyperkinesis. One major source of the variability in data among laboratories is the difference in the acoustic stimuli used by different investigators. Most have utilized highly complex stimuli, usually bells. These stimuli have at least three distinct characteristics: duration, intensity, and frequency. This study investigated the convulsability of DBA/2J mice of different ages challenged with a monotonic frequency using energy of about 60 to 65 dB. Most convulsions were observed between 18 and 21 days of age, though severe agitation was noted as early as 14 and as late as 35 days. Preliminary data indicate maximal responsiveness to monotonic stimulation around 20 to 25 kHz. Supported in part by USPHS Grant RR05423 to the UTCHS.

380 PENICILLIN-INDUCED ACTIVITY IN HIPPOCAMPAL SLICES MAINTAINED IN VITRO. Philip A. Schwartzkroin and David A. Prince. Dept. Neurol., Stanford Univ. Sch. Med., Stanford, CA. 94305.

Field potentials and neuronal activities in guinea pig hippocampal slices were studied after introduction of a convulsant drug (penicillin) into the bathing medium. Concentrations of penicillin as low as 1.7 mM induced spontaneous and evoked epileptiform field potentials. These complex waves were composed of multiple negative peaks and were almost identical to those recorded in vivo in hippocampal penicillin foci. The "burst" potentials were seen in areas CA1 and CA3 of the slice, but not in the dentate granule cell region. Epileptiform waves in CA1 followed those in CA3 with variable latencies. A cut made between CA1 and CA3 caused spontaneous bursting to disappear in CA1 but not in CA3; some bursting could still be elicited in CA1 by afferent stimulation. Increased Mg^{++} and decreased Ca^{++} concentrations abolished epileptiform discharge in both CA regions, thus demonstrating a dependence on synaptic activity. In single neurons, penicillin caused bursts of action potentials which coincided with peaks in the field potentials. Intracellular records showed that spike bursts could be triggered from a variety of membrane levels. In some cells, bursts appeared to arise from fast pre-potentials, while in other cells they were triggered from large depolarization shifts. The range of values for cell resistance and time constant was similar in neural populations from normal and drug-treated slices. The most obvious change in cells studied in the interval between normal and epileptogenic activity was an increase in depolarizing after-potentials. Our data suggest that potentiated dendritic activity, as reflected in fast pre-potentials and depolarizing after-potentials, may be a factor in generation of the epileptiform bursts.

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381 PENICILLIN-INDUCED MEMBRANE CONDUCTANCE CHANGES IN CAT SPINAL MOTONEURONS. P.C. Schwindt*, W.E. Crill. VA Hospital and Depts. of Physiology and Biophysics, and Medicine, School of Medicine, University of Washington, Seattle, WA, 98195.

Penicillin (PCN) applied topically to the cat spinal cord results in sudden depolarization shifts with high frequency spike bursts in many motoneurons. Under the condition of applied PCN, motoneuron somata have been subjected to voltage clamp analysis using independent current and voltage microelectrodes. Motoneurons exhibiting spike bursts and spontaneous firing after PCN application show an N-shaped, steady-state, current-voltage (I-V) relation in contrast to the concave-upward relation of normal cells. The negative slope portion of the I-V curve may descend below the zero current line indicating the presence of a steady inward ionic current. The subthreshold membrane potential during the depolarization shift corresponds to the negative slope portion of the I-V curve, suggesting the negative slope is causal in maintaining the depolarization shift and bursting need not be sustained by underlying synaptic currents. The steady inward current responsible for the negative slope portion of the I-V curve is not an inward potassium current caused by a shifted potassium equilibrium potential, but is rather a separate current component which is also present in less exaggerated form in some normal cells. Another fraction of the motoneuron population shows neither bursting nor abnormal I-V relations after PCN application. (Supported by VA Research Grant MRIS 1610)

382 PREDICTION OF PETIT MAL SEIZURES BY EEG POWER-SPECTRUM SIGNALS. Armand Siegel*, Physics Dept. & Sch. Med., and Allan F. Mirsky, Sch. Med., Boston Univ., Boston, MA. 02118

The work of Y. Takanobu (Fol. Psychiatr. et Neurol. 19:130, 1965) on the EEG spectra of patients with epilepsy has revealed in some cases an increase in power in the 3-Hz and 6-Hz regions immediately prior to seizure. We have examined background EEG in two patients with petit mal for reliable signals of approaching seizure. Such a signal must be (1) defined with quantitative precision, (2) reasonably free of interference by artifacts or rhythms uncorrelated with seizure, and (3) statistically reliable. Property (2) may require the steering of a narrow course between low-frequency artifacts on one side (which swamp the 3-Hz and 6-Hz regions) and the alpha rhythm on the other. We have devised a set of rules for visual scoring of a 9-Hz signal which, in one patient correlates with approaching seizure at $p<0.0001$. Spectra are calculated by the LINC spectrum program, for three consecutive 17.5 second intervals just before seizure, and for other time regions used as controls. At the present stage scoring is done by eye, with mental calculation. The choice of 9 Hz was dictated by the presence of large amounts of fluctuating seizure-uncorrelated power in the region 0-6 Hz, along with an alpha frequency clearly distinguishable from the 9-Hz region. The other patient has much more abnormal and irregular background EEG, but preliminary results also suggest prodromal 9-Hz activity. (Work supported by NIH Grants NS-12201 and MH-K5-14915).

383 AGE-DEPENDENT CHANGES IN THE EFFECTS OF SEIZURES ON THE DEVELOPING RAT BRAIN. N.J. Starr*and D. Holtzman*(SPON: R. Clayton) Dept. Neurology Sch. Med., Stanford U., Stanford, CA. 94305

A frequent pathologic finding after prolonged seizures in young children is cerebral edema (Lennox-Buchthal, Electroenceph. Clin. Neurophys. Suppl. 32: 60, 1973). In order to study the effects of seizures in the immature brain, a model was utilized which had previously shown nerve cell loss after repetitive seizures in 6-11 day rats (Wasterlain and Plum, Arch. Neurol. 29: 38, 1973). Rat pups between 2-30 days of age received 2 electroshocks (150V., 1 sec, AC) for 5-day periods to examine the effects of repeated seizures on brain weight and water content, body weight, and brain morphology. Between 11-15 days after birth, seizures produced the largest attenuation in body and brain weight gain, with no observable recovery. The maximal increase in brain percent water occured in 16-20 day old pups, which were exposed to seizures between 6-10 or 11-15 days of age. Correlated with changes in the percent of brain water were changes in electrolyte levels and neuroanatomical evidence of edema.

Our results indicate that repetitive seizures, during a critical developmental period, result in brain edema in the rat pups. The age-dependent presence of edema after seizures may be secondary to the limited capacity of the developing brain for energy metabolism (McIlwain and Bachelard, Biochemistry and the Central Nervous System, 1971). Cellular edema, as an inhibitor of mitochondrial respiration (Holtzman et al., J. Neuropath.Exp. Neurol., in press), may further depress brain energy metabolism, and be important in the genesis of post-seizure encephalopathy in the developing brain. (Supported by NIH Research Grants, NS 12151 and ES 01197 to D.H. and IT 32NS-02012-01 to N.S.).

384 INJECTIONS OF COBALT IONS INTO SPINAL MOTONEURONS. G. W. Sypert and W. D. Bidgood, Jr.*, Div. of Neurosurgery and Dept. of Neuroscience, University of Florida College of Medicine, Gainesville, Fla. 32610.

Recently, pial surface iontophoresis of cobalt ion (Co^{++}) has been shown to induce an epileptogenic focus. In order to clarify the physiological effects of Co^{++} on mammalian neurones, experiments were performed in cats under pentobarbital anesthesia. Varying concentrations of Co^{++} were injected inside lumbosacral motoneurones by electrodiffusion. Histological verification of the intracellular location of Co^{++} was made using light microscopy. There was a long-lasting depolarizing shift of the IPSP-reversal potential (E_{IPSP}) towards the resting potential with a reduction in the conductance increase normally associated with the IPSP (g_{IPSP}). Intracellular Co^{++} probably did not alter the g_{Cl} increase of the IPSP. Hence, it was concluded that the fall in E_{IPSP} and g_{IPSP} caused by intracellular Co^{++} is mainly due to a decrease in g_K. In addition, there was a long-lasting increase in motoneuron excitability (decrease in rheobase to injected currents). Resting potential, membrane resistance and action potentials (including the differentiated records) were not consistently altered by intracellular Co^{++}. It is suggested that Co^{++} acts on specific postsynaptic membrane sites to reduce inhibition (disinhibition) and increase all or none membrane excitability which accounts for its epileptogenicity.

385 STATUS EPILEPTICUS: A NEW RODENT MODEL. Katherine H. Taber*, John J. McNamara*, and Steven F. Zornetzer (SPON: B.J. Wilder). Dept. Neurosci., College of Medicine, U of Florida, Gainesville, FL. 32610.

Kindling, the phenomenon of developing a persistant seizure-prone area of brain by administering repetitive low intensity electrical stimulation, has recently undergone intensive investigation. The most commonly used interstimulus interval has been 24 hours. Using an interstimulus interval of one minute we have found that it is possible to produce longterm self-sustained seizures (SSS) in mice via hippocampal electrode placements. These results suggest an animal model for the clinically defined syndrome of status epilepticus.

Effective stimulus parameters for the production of SSS were: 400uA current intensity(constant current), 1 msec bipolar square wave pulses, 60 Hz, 1 sec train, 1 train/trial. With electrodes located in hippocampal subfields CA2, CA3, and CA4 and in the dentate gyrus a slowly developing form of SSS were seen. This form of seizure was characterized by a slow (100 to 130 stimulations) increase in the intensity and duration of after-discharge. Behaviorally the major manifestations were automatisms or stereotyped behaviors(repetitive grooming, chewing, etc.) and behavioral arrest. These animals were deficit on a one-trial passive avoidance task 24 to 48 hours after the cessation of SSS.

Electrode placements in CA1 of hippocampus generally resulted in a more quickly(40 to 50 stimulations) developing form of SSS. Behaviorally these mice exhibited both preconvulsive and convulsive motor activities. All these animals died in convulsion within one hour of the onset of SSS.

The first form of SSS (Non Lethal SSS) is discussed as a model for psychomotor status epilepticus. The second form (Lethal SSS) is discussed as a model for convulsive status epilepticus.

386 EFFECT OF CORTICAL ABLATIONS ON RETICULAR MULTIPLE UNIT DISCHARGES DURING PENTYLENETETRAZOL SEIZURES. Francisco Velasco, Marcos Velasco and Héctor Maldonado*. Sci. Res. Dept. National Medical Center, I.M.S.S. Mexico City, Mexico.

The present investigation studies the increments in multiple unit activity of the mesencephalic reticular formation (RFMUA) after pentylenetetrazol (PTZ) injection in cats paralyzed with gallamine. In these cats, cortical ablations were done 30 days before the experiment and their results were compared to those obtained from intact cats in order to elucidate if cerebral cortex normally modulates the threshold, duration and intensity of PTZ seizures. Animals with ablation of the primary cortices (visual, auditory and somesthetic) significantly increase PTZ threshold and decrease duration and intensity of RFMUA. In contrast, animals with ablation of the associative cortices (parietal, cingulate, prefrontal and orbito frontal) significantly decrease PTZ threshold and increase duration and intensity of RFMUA. These results suggest that in intact animals primary cortices exert a facilitatory while associative cortices exert inhibitory tonic influence on PTZ seizures.

387 WAKEFULNESS-SLEEP MODULATION OF PYRAMIDAL TRACT DISCHARGES FROM CHRONIC EPILEPTOGENIC CORTICAL FOCI IN CATS. Marcos Velasco, Francisco Velasco, Carlos Cepeda* and Francisco Estrada-Villanueva. Natl. Med. Ctr. I.M.S.S. México, D. F.

Injection of a critical amount of alumina cream into the motor cortex of cats produces a predictable model of clinical and EEG focal motor seizures. Cortical EEG spikes (CES) are consistently accompanied by muscle twitching when animals are awake but CES appeared in absence of muscle twitching when animals are asleep. We studied here the temporal relationship between onset of CES and increased frequency of pyramidal tract multiple unit activity (PTMUA) during wakefulness and sleep of these epileptic cats. We have found that CES is accompanied by a significant transient increase PTMUA during wakefulness and paradoxical sleep while CES is not accompanied by significant changes in PTMUA during slow wave sleep. These data suggest that presence or absence of clinical convulsions during wakefulness and slow wave sleep depends upon a modulatory intracortical mechanism while absence of clinical convulsions during paradoxical sleep depends on an inhibitory spinal mechanism.

388 EPILEPTIGENIC EFFECT OF SUPRACORTICALLY-APPLIED PROSTAGLANDIN (PG) E_1 FOLLOWING PRETREATMENT OF RABBITS WITH PG SYNTHETASE INHIBITORS. Martin C. Wallenstein and Laszlo Z. Bito . Dept. Ophthal., College of P & S, Columbia Univ., New York, N.Y. 10032.

Following chronic implantation of supradural electrodes and a subdural cannula over the visual cortex, suprafusion of 8 μg PGE_1 in 125μl saline produced no observable effect on spontaneous electrical activity or on visually-evoked responses (VER). The same amount of PGE_1 suprafused 10 to 30 min. after the IV or IP administration of high doses of PG transport inhibitor, probenecid or indomethacin, produced epileptiform activity. These results are consistent with the concept that inhibition of PG transport across the blood-brain barrier results in the retention of the suprafused PGE_1 levels within the brain tissue.

Pretreatment with a low concentration of indomethacin (10 mg/kg I.V.) which is expected to inhibit PG synthesis but not PG transport, also rendered the cortex sensitive to these effects of PGE_1 but only after a delay of at least 2 hrs. Tremors, convulsive and/or coordinated running movements were noted in most animals during the PGE_1-induced epileptiform activity. Pretreatment with paracetamol (50 mg/kg I.V.), a PG synthetase inhibitor with much weaker effects on PG transport, yielded similar results. These findings are interpreted as indicating that inhibition of PG synthesis results in the lowering of the concentrations of endogenously produced PGs (including the very abundant but less potent $PGF_{2\alpha}$) which, in turn, may lead to elmination of competition with the exogenous PGE_1, or to the development of true supersensitivity. The possible role of PGs in epilepsy is of special interest since, unlike other epileptigenic substances, PGs are produced within the brain normally, and in increased amounts under pathological conditions, including epilepsy.

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389 EFFECT OF LYSERGIC ACID DIETHYLAMIDE ON KINDLED SEIZURES. John G. Zoll, David A Kovacs* and Daniel T. Linehan*
Dept. Neurosurgery, Sch. Med., SUNY, Buffalo, N.Y. 14215

In order to obtain more knowledge about median raphe nucleus d-lysergic acid diethylamide (LSD-25) was injected intra peritoneally into kindled rats. In one-half of these animals a lesion was made in the median raphe nucleus. LSD-25 elevated the threshold for seizures in the rats with intact raphe nucleus but not in those where the median raphe nucleus had been destroyed.

This result is further evidence that 5-hydroxytryptamine (5-HT) which is synthesized by cells in the median raphe nucleus has a suppressive influence on kindled seizures.

Extraocular Movements

390 EFFECTS OF A CONTINUOUSLY MOVING ENVIRONMENT ON THE RHESUS MONKEY'S OCULAR STABILITY IN THE DARK. F.A. Miles. Lab. Neurophysiology, NIMH, Bethesda, MD. 20014.

Recent work has shown that optical disturbances of the visual input associated with head movements can induce long-term adaptive changes in the vestibulo-ocular system (1-3). The present experiments attempted to introduce a right-left asymmetry into this system by exposing animals to an environment moving continuously in one direction. Two monkeys were subjected to a moving environment by seating them inside a continuously rotating striped drum (speed 13°/sec as seen by the animal) and when, after several hours exposure, they were subsequently placed in total darkness, a slight ocular drift was immediately evident; this developed into a pronounced nystagmus over a 1-2 min period (slow phase velocities reaching 50°/sec after 24 hours inside the drum) with the slow phase always opposite in direction to the original stripe movement. It required up to 24 hours of exposure to a stationary environment to completely abolish this dark nystagmus. The same effects were seen whether the head was free or fixed during any part of the procedure, provided that the exposure to the moving stripes lasted no more than a day or so; after longer periods inside the rotating drum the effect of the subsequent exposure to a stationary environment depended on the freedom of head movement. Thus, if the head had been free to move over a period of several days inside the moving drum and was then fixed for 24 hours with the drum stationary, a dark nystagmus was still evident, but now reversed in direction (slow phase velocities, 5°/sec): the eyes were now drifting in the same direction as the original stripe movement. Oscillating the animal increased the drift rate to 15-25°/sec. This reversal was not seen if the animal's head had been fixed while viewing the moving stripes and hence is assumed to represent an adaptive bias in the vestibulo-ocular system. The vestibulo-ocular responses of the two monkeys were assessed by measuring their compensatory eye movements during passive oscillation about a vertical axis in the dark. This was accomplished with a servo-driven chair powered by a torque motor under the control of a waveform generator. The monkeys' heads were secured to the chair in this test situation through implanted bolts and their vestibular-ocular reflex gains (assessed as peak-to-peak eye velocity/peak-to-peak chair velocity) were always in the range 0.9-1.0 throughout all of these procedures; the compensatory eye movements were simply superimposed on the dark drift (which was evident as a drift in gaze during oscillation). The initial pronounced dark nystagmus with the slow phase always opposing the original stripe movement is assumed to be an oculomotor manifestation of some adaptive process in the visual system (cf., the waterfall phenomenon); the postulated vestibular bias is evident only after the visually induced phenomenon has been abolished by fixing the head in a stable environment.

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391 MORPHOLOGY OF MOTORNEURONS AND INTERNEURONS IN THE CAT ABDUCENS NUCLEUS.
R. F. Spencer and P. Sterling. Dept. Anat., Univ. Penn. Sch. Med., Philadelphia, PA. 19174.

Neurones in the cat abducens nucleus which innervate the lateral rectus muscle and those which project to the oculomotor nucleus have been identified at the electron microscope level utilizing the retrograde intraaxonal transport of horseradish peroxidase (HRP). HRP-labelled neurones were identified in 1 μm sections at the light microscope level and studied by electron microscope examination of adjacent ultra-thin sections. Samples for electron microscope analysis were obtained at approximately 5 μm intervals throughout the extent of the nucleus. Quantitative measures were obtained from neurones which had been sectioned through the nucleus and nucleolus. Following injections of HRP into the lateral rectus muscle, motoneurones in the ipsilateral abducens nucleus, ranging in size from 10 μm to 42 μm in diameter, with peaks at 16 μm, 24 μm, and 34 μm, were labelled. The motoneurones characteristically contained stacked arrays of rough endoplasmic reticulum, in the larger neurones often arranged in extensive and elaborate networks. The large motoneurones had round, non-invaginated nuclei with regular nuclear membranes. Smaller motoneurones had a tendency to have elliptical nuclei with fluted or invaginated nuclear membranes. There was a wide variation between motoneurones in the density of synapses on the somatic surface, ranging from 0.23 to 17.64 synapses/100 μm^2. The ratio of synapses containing spheroidal vesicles to synapses with flattened vesicles (S/F ratio) also varied widely from cell to cell, ranging from 0.45 to 4.17. The values of these two measures were not correlated with the size of the motoneurone, nor were they correlated with each other. Thus, the physiological differences between abducens motoneurones, such as order of recruitment, may not depend solely on differences in anatomical size, but may also be a function of differences in other parameters, such as synaptic density and S/F ratio on the motoneurone surface.

In contrast to spinal motor nuclei, no axo-axonic synapses have been observed. There was also no evidence for the existence of gap junctions within the area of the nucleus studied, despite the physiological evidence for electrotonic coupling of the motoneurones. Although motoneurones are frequently arranged in clusters in the central portion of the nucleus, specialized appositions between adjacent neurones or their proximal processes have not been observed.

Following multiple microinjections of HRP along the rostro-caudal extent of the oculomotor nucleus, labelled neurones in the contralateral abducens nucleus resembled the neurones previously identified as motoneurones, both in the range of sizes (10 μm to 34 μm) and in cytological characteristics, particularly the cisternal arrays or rough endoplasmic reticulum. Although there was a tendency for labelled neurones to be located in the dorsal and lateral portions of the nucleus, it was common to find labelled neurones intermingled with unlabelled cells throughout the nucleus. These observations raise the possibility that the afferent projection to the oculomotor nucleus from the abducens nucleus may not arise from a special class of "interneurones," but as collaterals of some of the abducens motoneurones themselves.

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392 NONCONTACTING, HORIZONTAL AND VERTICAL EYE MOVEMENT MONITOR

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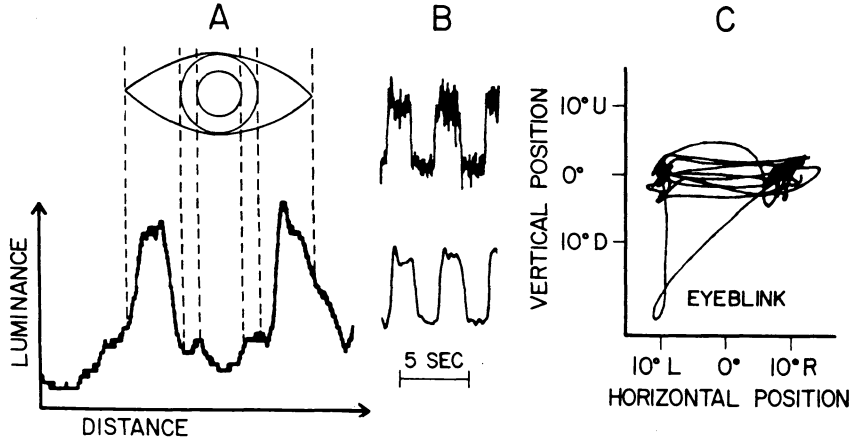
This computer controlled image dissector camera for recording eye movements requires no mechanical or electrical contact with the subject. Pupil position is monitored by tracking the region of lowest luminance, as shown by the reflected light profile obtained from a horizontal eye scan (Fig. A). Algorithms were also developed to track horizontal and vertical pupil-iris boundaries, enabling pupil center computations 40 times per second with a resolution of 0.5 degrees. Figure B shows horizontal eye position as a function of time before (top) and after (bottom) lowpass digital filtering for saccades between two targets located 20 degrees apart. The horizontal and vertical eye position data were processed separately with digital filters designed to provide low noise records and a bandwidth large enough to allow study of saccadic shapes. Figure C shows a plot of vertical versus horizontal eye position for 9 successive saccades between the targets. Eyeblinks were identifiable by their characteristic trajectory (Fig. C) resulting from tracking of an artifactual dark area (e.g. an eyelash) followed by automatic return to pupil tracking. Our technique provides significant advantages over other eye movement recording methods: low noise, freedom from drift, and automatic, objective calibration of absolute eye position. Other algorithms are being developed for enhanced speed and resolution, and monitoring relative eye versus head position.



393 VESTIBULAR PROJECTION TO MEDIAL RECTUS MOTONEURONS IN THE CAT. R. Baker and S.M. Highstein. Div. of Neurobiol., Univ. of Iowa, Iowa City, Ia. 52242 and Dept. of Neuroscience, Kennedy Center for Research, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Synaptic events in medial rectus motoneurons (MR Mns) were recorded following electrical stimulation of the peripheral and central vestibular complex in the anesthetized cat. Activation of the ipsilateral vestibular nerve (Vi) produced a large (ca. 5mV amp), disynaptic EPSP with a latency of 1.4 msec in MR Mns. Stimulation of the contralateral vestibular nerve (Vc) produced a small (<.5mV amp), disynaptic (1.4msec) EPSP in the same MR Mns. In most experiments, a small amplitude, long latency (3-4 msec) inhibition followed the Vc evoked EPSP. Surprisingly, we never observed a Vc disynaptic inhibitory pathway comparable to that demonstrated for all other ocular Mns. Furthermore, central stimulation of the MLF, vestibular and abducens nuclei produced only short latency excitation (EPSPs not IPSPs) in MR Mns thereby indicating the likely absence of any inhibitory neuron in the ponto-medullary brain stem. By employing various midline and coronal lesions (both acute and chronic) we found the powerful Vi excitatory pathway to ascend lateral to the MLF in the pontine tegmentum. In fact, its central course corresponded well to the "ascending tract of Deiters" which, anatomically, has been described to originate in the ventral part of the lateral vestibular nucleus and ascend to the MR subdivision of the oculomotor complex. Thus, we conclude that the MLF contains neither direct inhibitory nor excitatory vestibular projections to MR Mns. These results support the concept that reciprocal organization for conjugate horizontal gaze is primarily effected by internuclear neurons of the abducens nucleus (see abstract by Highstein & Baker). Finally, we suggest that the absence of direct inhibitory vestibular neurons to MR Mns may be linked with the necessity for maintaining different accommodative levels (vergence) during conjugate gaze. Supported by PHS Grants EY-01074, EY-00003 and EY-01670.

394 HOW THE EYE KNOWS WHERE THE WORLD IS. Bruce Bridgeman and Stephen J. Lewis*, Psychology Board of Studies, U. of Ca., Santa Cruz, CA, 95064

Subjects are unable to detect the rapid displacement of a target when the displacement occurs near the time of a saccadic eye movement. This poses a paradox: how can visual-motor coordination be maintained after a saccade if information about the absolute position of the world is degraded during the saccade? The paradox could be resolved by hypothesizing that two levels of the visual system have access to different degrees of spatial information, so that motor-oriented activities might have access to accurate absolute position information even when that information is not available at a perceptual level. The perception-of-displacement experiment accesses only the perceptual system.

We tested this hypothesis by asking a subject to point to the center of the stimulus display with an unseen pointer, following either a detected displacement or an undetected one. At unpredictable times the stimulus was rapidly displaced from a left position to a position 2° to the right or vice versa. Eye movements were measured with a photoelectric technique, and all experimental parameters were digitized and recorded directly in computer core. Pointing when the stimulus was objectively on the left was significantly different ($p < .01$) from pointing when the stimulus was on the right, whether or not the displacement from one position to the other had been detected. In a second experimental condition the stimulus was extinguished before pointing, forcing the subject to use internal spatial coordinates rather than any image-related cues to determine direction. These results can resolve several seeming discrepancies in the literature on eye movements and space perception.

395 CONVERGENCE OF EXTRAOCULAR AND DORSAL NECK MUSCLE AFFERENTS TO AREAS CORRESPONDING WITH THE FRONTAL EYE FIELDS (FEF) IN THE CAT. B. Dubrovsky and H. Barbas* Neurophysiol. Labs, Allan Memorial Institute, McGill University, Montreal, Quebec (SPON: Dr. J. Gordon).

Previous studies (Dubrovsky and Barbas, Neurosc. Abs. 361, 1975) have shown that stimulation of dorsal neck muscle afferents, electrophysiologically characterized as belonging to group I fibres, evoked short latency (6-10 msec) neuronal activity in the frontal eye field areas of the cat. These responses were abolished after dorsal column section. Since behavioral investigations (Exp. Brain Res. 18, 165, 1973) showed significant deficits in tracking in a sequential motor act following dorsal column section, we suggested that information from dorsal neck muscles coursing through the dorsal columns is necessary for precise eye-head tracking movements when the head moves in relation to the body while the body is in motion. We proceeded to investigate whether proprioceptive stimuli from extraocular muscles project to the same frontal eye field areas that receive information from dorsal neck muscle afferents.

Electrical stimulation of the nerves of the superior rectus and superior oblique muscles elicited short latency (10-12 msec) field and single cell activity in the FEF areas. Moreover a high degree of convergence was observed at the single cell level between dorsal neck and extraocular muscle afferents. This convergence was significantly higher than the convergence observed between the rectus capitis posterior major, and obliquus capitis inferior (deep muscles) as well as the biventer cervicis, and splenius muscles. The convergence of extraocular and dorsal neck muscle afferents onto FEF areas suggests an involvement of these cortical areas in mechanisms related to eye-head movements.

396 SACCADIC AND SMOOTH PURSUIT EYE MOVEMENTS OF THE CAT. L.C. Evinger* and A.F. Fuchs, Dept. Physiol. & Biophysics, and Regional Primate Research Center, University of Washington, Seattle, WA 98195.

Although in recent years both anatomical and electrophysiological studies have begun to reveal the connectivity of brainstem oculomotor structures in the cat, very little is known about normal feline eye movements. In order to determine how cats follow moving targets we have measured their eye movements with a magnetic search coil and rewarded them for accurate tracking. The results indicate that qualitative and quantitative differences exist between primate and cat eye movements. In order to acquire a target that stepped eccentrically from the primary position, cats usually employed saccades. Saccades to eccentric targets were usually less than 20 deg. and rarely exceeded peak velocities of 350°/sec. Horizontal saccade amplitude and duration were best related by a regression line of slope 5 msec/deg. Vertical saccade amplitude and duration were linearly related by a line of slope 5 msec/deg. Saccadic trajectories exhibited considerable variability even among saccades of equal amplitude. Saccades often maintained a constant velocity during part or all of the saccade. Cats employed smooth eye movements of 20-30 deg/sec, though less frequently than saccades, to acquire these eccentric targets. Smooth pursuit eye movements were studied by using ramp and sine wave target movements. The velocity of horizontal smooth pursuit movements elicited by target ramps was linearly related to target velocity only up to 8 deg/sec in either direction; for higher target velocities, eye velocity never exceeded 8 deg/sec. Consistent with the ramp data, the gain of smooth pursuit eye movements in response to sinusoidal target movements was down by 3 db at a peak velocity of 6°/sec irrespective of target amplitude or frequency. Vertical smooth pursuit reached peak velocities of 5 deg/sec in the upward direction and 2 deg/sec in the downward direction.

397 SACCADES ACCORDING TO INSTRUCTIONS. Peter E. Hallett. Dept. Physiology, University of Toronto, Ontario M5S 1A8 Canada.

The subject fixated the target spot, when it was lit, with her left eye. The spot then stepped at random, L or R, to 1 of 8 equally likely positions in the range ± 15 deg, but the subject's goals depended on her instructions - which differed in different sessions.

The hyposaccade (Ho)instruction is "Make an eye movement equal to 0.5 of the target step". The hyper-saccade (He) instruction is to make an eye movement a fixed amount larger (by 3.8 deg) than the step. The anti-saccade (A) instruction is "Make an eye movement equal and opposite to the target step". Normal saccadic tracking (N) serves as a control condition.

She performed these tasks on the first or second attempt. The movements consisted of 1 or 2 saccades. Primary saccade amplitude versus duration was normal. Latency and errors increased in the order N, (He and Ho nearly equal), A. Certain aspects of the He, Ho and A data showed L/R (abduction/adduction) asymmetry. Corrective saccades followed the primary saccade with reduced frequency, increased size and typical latency, and tended to reduce the error between eye and goal.

The N and A expts have been repeated on a male of similar age with biofeedback of latency and error. His results differ from the above in several ways, but A latency is prolonged and A errors are quite gross.

"Foveation" is quicker, more precise and more consistent than peripheral placement of the retinal image.

398 TERMINATION OF INTERNUCLEAR NEURONS OF THE ABDUCENS NUCLEI ON MEDIAL RECTUS MOTONEURONS. S.M. Highstein and R. Baker. Dept. of Neuroscience, Kennedy Center for Research, Albert Einstein College of Med., Bronx, N.Y. 10461 and Div. of Neurobiol., Univ. of Iowa, Iowa City, Ia. 52242.

Projections from neurons within the abducens nucleus to the contralateral medial rectus (cMR) subdivision of the oculomotor complex were studied with intracellular recording in anesthetized cats. Ipsi- (Vi) and contralateral (Vc) vestibular nerves were stimulated and concentric stimulating electrodes were placed in the abducens nucleus (AbdN). Following AbdN stimulation (2-3x Thr) field potentials in the cMR subdivision showed a sharp positive-negative presynaptic transient followed by a slower postsynaptic negativity. In identified cMR Mns, stimulation of the AbdN produced mono- (ca. 0.6msec) and polysynaptic (>1msec) EPSPs. These EPSPs were of similar amplitude to those produced by Vi stimulation (see following abstract by Baker & Highstein) and both depolarizations were reversed by current injection through the microelectrode. To determine the uniqueness of this internuclear pathway the AbdN was separated from the ipsilateral vestibular and rostral pontine reticular nuclei by sagittal and coronal lesions in two cats. A third cat had coronal sections rostral and caudal to the AbdN and a fourth one had a coronal cut behind both AbdNs including a sagittal cut isolating the contralateral vestibular nuclei. Acute electrophysiology was performed 4-5 days subsequent to the lesions in these 4 chronic cats and the findings with respect to projections from the AbdN to the cMR Mns were identical to control experiments. We conclude that the internuclear neurons previously identified within AbdN terminate exclusively with an excitatory synaptic action upon cMR Mns. We suggest that this internuclear projection is important in the organization of conjugate horizontal gaze and its interruption is directly responsible for the deficits in horizontal eye movements observed clinically in the syndrome of internuclear ophthalmoplegia. Supported by PHS Grants EY-00003, EY-01670 and EY-01074

399 GAIN OF THE VESTIBULO-OCULAR REFLEX IN MONKEY AT HIGH ROTATIONAL FREQUENCIES. Edward L. Keller. The Electronics Research Lab., Univ. of Cal., Berkeley, CA 94720.

The gain of the vestibulo-ocular reflex (VOR) was measured in alert monkeys during sinusoidal oscillations of the animals' heads about a vertical axis over a frequency range extending from 0.5 Hz to 6 Hz. Measurements were made first in total darkness and then in a normally lighted room with the animals attending to an earth-fixed visual target display. In confirmation of the results of previous investigators the gain of the VOR was found to be consistently below unity (mean of 0.87 in four animals) in the dark at rotational frequencies up to 1 Hz. When visual information was present to augment the vestibular input the gain increased to a value of one in all four animals over this same frequency range. In contrast, as the rotational frequency increased above 1 Hz, a range not previously investigated, the gain in the dark increased steadily until it exceeded unity in all four animals at 2 Hz. Peak gain (mean = 1.3) was reached in all animals in the dark at about 4 Hz and declined sharply at higher frequencies to fall below one at 6 Hz. Gain in the presence of fixed visual images remained at one over this entire range of frequencies. These results have important implications in relating the discharge of single neurons in the brain stem and cerebellum of these same animals to the generation of the VOR. If one supposes that inputs from directionally selective visual slip detectors are combined with the pure vestibular drive to produce the unity gain observed in the VOR in the presence of visual images, then one would expect a different set of visual slip detectors to become activated in the frequency range above 2 Hz where the eye velocity in the presence of exclusive vestibular input would actually exceed head angular velocity.

400 SMOOTH TRACKING WITH COMBINED EYE-HEAD MOVEMENTS. Jeremy M. Lanman*, Emilio Bizzi and John Allum*. Dept. Psychology, M.I.T., Cambridge, Mass. 02139.

In this series of experiments we investigated the coordination of eye and head movements during smooth pursuit of a visual target. Monkeys were trained to make a visual discrimination between a horizontal and a vertical bar (3 min.in width) which were projected on a screen and moved smoothly at a frequency between .1 to 1.0 Hz. Generally the animals tended to follow the target with the head and kept their eyes near the center of the orbit. Often the head movements were found to be quite irregular--however despite this, the gaze (i.e. the sum of eyes and head movements) was remarkably on target. Close observation of the phase relationship between the moving visual stimulus and the gaze indicated that the monkeys could follow the target either in a "predictive" mode (i.e. with no measurable delays) or in a servo mode. In the latter case, small saccades at approximately 300 msec. intervals were seen. In order to gain some understanding of the mechanisms underlying these modes of coordination we stopped unexpectedly the head movement during tracking. We found that the eyes compensated for the head deceleration with an equal and opposite acceleration. This compensatory acceleration required only about 20 msec. and, remarkably, resulted in a negligible gaze error. These results and others in which tracking of vestibulized monkeys were analized are consistent with the assumption that there is an internal representation of target motion in space. (Research supported by NIH grant NS09343 and NASA grant NGR 22-009-798.)

401 PULVINAR UNIT RESPONSES ASSOCIATED WITH EYE MOVEMENTS IN SQUIRREL MONKEYS. Kent M. Perryman*, David F. Lindsley and Donald B. Lindsley. Depts. of Psychol., Physiol., Psychiat. and Brain Res. Inst., UCLA, Los Angeles, CA. 90024 and Dept. Physiol., USC Sch. Med., Los Angeles, CA. 90033.

Extracellular microelectrode recordings were obtained in the pulvinar nuclei of 4 chronic, restrained squirrel monkeys. The monkey sat with its head centered in a translucent milk-plastic hemisphere providing a Ganzfeld (homogeneous illuminated field). The head was fixed but eyes were free to move. Repeated penetrations over days sampled all regions of the pulvinar. Post-stimulus-time histograms (PSTHs) were triggered at onset of horizontal saccadic eye movements by extraoculograms from electrodes located at external canthi or by a light flash. PSTHs were compared under three conditions: lighted Ganzfeld, dark adaptation, light flash in the dark. About 10% or 150 cells showed eye movement responses (EMRs) in the light, whereas less than 1% or 12 exhibited EMRs in both dark and light. 50% of light and dark EMR cells responded to light flash. Both excitatory and inhibitory patterns were found. Medial, lateral and inferior pulvinar regions were studied, but only those cells in a narrow strip along the margins of the medial and lateral pulvinar were responsive to eye movements. This region in the rhesus monkey has been identified by Benevento and Fallon (J. comp. Neurol. 160: 339-361, 1975) as receiving projections from the deep layers of the superior colliculus where cells respond to eye movements and where eye movements can be elicited by electrical stimulation (Schiller and Stryker, J. Neurophysiol. 35: 915-924, 1972). Our data show that many units at the border of the medial and lateral division of the pulvinar respond to both eye movements and light flashes, suggesting that this region of the pulvinar is involved in the processing of both oculomotor and visual information and may play a role in visual attention. (Supported by USPHS grant MH-25938 and by the Grant Fdn.)

402 QUANTITATIVE MODELING OF OPTOKINETIC AFTER-NYSTAGMUS (OKAN) GENERATION.

Theodore Raphan, Victor Matsuo and Bernard Cohen*, Mount Sinai School of Medicine and City College, of CUNY, New York, 10029.

OKAN was studied in alert monkeys stimulated with constant velocity whole field rotation for varying durations, and a simple model of OKAN generation was formulated which explains the more prominent aspects of the response. OKAN is fully developed after 3-5 sec of OKN but declines more slowly over 25-50 sec. The rapid rise and slow decay of OKAN slow phase velocity is analogous to the behavior of a "peak" detector which was used as the basis of the model. We postulate a central integrator with a 10-20 sec time constant (T_c) which "charges" through a high feed forward gain during OKN. This integrator then "discharges" at its own characteristic T_c while driving the eyes during OKAN. It was predicted that OKAN peak velocity would be a fixed percent of stimulus velocity for stimulus durations above 5 sec. This was found to be correct for stimulus-velocity ranges up to 120°/sec. Saturation occured above this level. Visual fixation of more than 2-4 sec abolishes OKAN and was explained by an additional feedback element which discharges the integrator rapidly when fixation is permitted. Fixation times of less than 2 sec discharged the OKAN generator to levels predicted by the model. Other data suggest that the OKAN generator is the same mechanism which drives the eyes during post-rotatory nystagmus. The model provides a theoretical framework for understanding how this mechanism might be organized.

(Supported by NINCDS, NSF and CUNY Research Foundation.)

403 AN ELECTROPHYSIOLOGICAL STUDY OF CAT RETICULOVESTIBULAR NEURONS.
R. S. Remmel and R. D. Skinner, Physiology Dept. and Anatomy Dept., Univ. of Ark. for Med. Sciences, 4301 W. Markham, Little Rock, AR 72201.

In Nembutalized decerebellate cats, a stimulating electrode was placed into the left medial vestibular nucleus (MVN) where the antidromically-excited field potential from the right ascending medial longitudinal fasciculus (MLF) was large. Electrode locations were verified histologically. The medial pons and medulla were systematically surveyed with extra- and intracellular micropipettes for units excited antidromically from MVN. Such units were detected in the contralateral but not the ipsilateral parabducens nucleus immediately ventral to the abducens nucleus. Thus, in conjunction with our earlier results on ascending MLF projections, some parabducens neurons project into the contra.but not the ipsi. MLF and other parabducens neurons project toward the contra. but not the ipsi. MVN. MVN-projecting units were also found bilaterally in nucleus reticularis gigantocellularis and the ventral part of nucleus reticularis pontis caudalis. Thus, in conjunction again with our earlier results, some neurons in ventral caudalis and gigantocellularis project into the ipsi. MLF and others into the contra. MLF, while still others project toward the ipsi. MVN and others toward the contra MVN. Commissural neurons were detected in the contra. vestibular nucleus. Neurons projecting toward the MVN were not found, however, in either prepositus hypoglossi nucleus. Many of the above units seemed to be somas because they had primarily negative extracellular pulses of -0.05 to -0.2 mV amplitude and could be detected over at least 0.15 mm along the electrode track. These reticulovestibular neurons are expected to be involved in eye and head movement control. Units orthodromically-excited from the MVN and units excited from both the MVN and the MLF were also detected.

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404 THALAMIC UNIT ACTIVITY ASSOCIATED WITH VISUAL ORIENTATION IN CAT.
John Schlag and Madeleine Schlag-Rey*. Dept. Anat. and BRI, UCLA, Los Angeles, CA 90024.

It was previously shown that most (>90%) of eye movement-related units recorded in the thalamic internal medullary lamina (IML) of chronic cats were responsive to photic stimuli. The receptive field of these cells was always on the side toward which saccades were preceded by increased firing. IML units were studied in a behavioral situation where the animal could orient at any time after presentation of a faint photic target. Two peaks of firing could then be distinguished in individual trials: one related to stimulus onset, the other to the targeting saccade. When targeting occurred after a delay (sometimes more than 1 sec after stimulus onset), the activity signaling the appearance of the target died off long before the eye movement-burst was initiated. Thus, although the latter burst was causally related to the former unit response it must have been timed by another input.

The involvement of the cat's IML in goal-seeking activity is further substantiated by the observation of 16 units which fired only with targeting saccades. These units were presaccadic and direction selective; their activation depended on the presence of a target (at the time of the saccade or shortly before the saccade) and on the aiming of the eye movement toward this target.

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405 EFFECTS OF STRABISMUS SURGERY ON SPATIAL LOCALIZATION.
M.J. Steinbach* and D.R. Smith* (SPON: J.P. Landolt). York Univ., Toronto; Hosp. for Sick Children, Toronto, Canada.

Patients undergoing monocular surgery for horizontal strabismus (exotropia) were assessed, before and after surgery, on their ability to point with the unseen hand at a small light in a totally dark room. Viewing was always monocular and pointing was measured using the unoperated and operated eye in turn. The intent of the surgery was to correct for $12\frac{1}{2}$ to 15° of eye turn and the deviation was reduced by at least this amount. Following surgery, the operated eye was dressed in a manner that precluded any vision until the time of testing, 24 to 48 hrs later. Pointing responses were assessed using the operated eye the instant the bandage was removed and these showed average shifts (compared to the pre-op measures) of from 10% to 49% of the amount that should have resulted from the surgery. In addition, several patients showed post-op shifts when the unoperated eye was tested and these were in the direction opposite that which could have been expected from the surgery. A control subject, monocularly occluded for 48 hrs, showed only a trivial post-occlusion shift.

These results indicate that the oculomotor system had access to information about the position of the operated eye in its orbit before the bandage was removed. While the mechanism for recalibration remains unknown, information about eye position must have been obtained from proprioceptive (or inflow) sources.

Feeding and Drinking

406 THE EFFECT OF STRIATAL KNIFE CUTS ON EATING AND DRINKING. G.F. ALHEID*, J. Kelly*, L. McDermott*, A. Halaris, and S.P. Grossman. Dept. Beh. Sci. Univ. Chicago, Chicago, Ill. 60637

Vertical knife cuts were made at the lateral edge of the lateral hypothalamus (LH) or adjacent to the medial surface of the globus pallidus (GP) in male albino rats. Both cuts decreased striatal Dopamine (DA), but GP cuts were less effective than LH cuts (median, LH=20%, GP=45% control). Despite this difference, LH or GP cuts produced similar periods of aphagia (median, LH=5, GP=6 days) and adipsia (median, LH=6, GP=7 days). In contrast, coronal knife cuts just anterior to the GP produced only slight aphagia (median=1 day) eventhough caudate DA was only 10% of control levels. Striatal DA was correlated with ingestive behavior only in rats with LH cuts (DA x aphagia=-.81, DA x adipsia=-.74, DA x 24 hr water intake, when food absent=.71, n=17). LH cuts inhibited eating relative to control rats after i.p. insulin (4U) or 2-deoxy-glucose (2-DG)(750mg/kg) although significant increases were observed relative to baseline amounts. GP cut rats ate normally in response to insulin injection, but showed no significant increase in eating after 2-DG. Both LH and GP cut rats drank subnormal amounts of water after i.p. injections of 2M NaCl, or s.c. injections of 30% polyethylene glycol (PG). If LH or GP rats were given .15M NaCl to drink rather than water after PG injection, fluid intake of LH rats did not then significantly differ from controls. The intake of GP rats while slightly increased relative to previous water intake, remained subnormal relative to .15M NaCl intake by control rats. By 60 days post-op, body weights of rats with LH cuts did not significantly differ from controls, but that of GP rats remained chronically low, even 9 months after surgery.

Both LH and GP cut rats suffered enhanced anorexia relative to controls after Norepinephrine (NE) depletion by diethyldithiocarbamate, or after alpha-NE blockade by phenoxybenzamine. LH rats were slightly more anorexic than controls after i.p. haloperidol, but GP rats did not differ from controls at any dose tested.

These results suggest that: (a) DA depletion and destruction of non-DA striatal axons produce similar but not identical effects on ingestive behaviors, and that these effects may contribute additively to the LH lesion syndrome. This is consistent with the observations of Morgane (1961) on the effects of lesions in the GP. (b) DA depletions may have to occur in the presence of intact caudato-pallidal connections before eating and drinking are disrupted. (c) Peripheral injections of insulin and 2-DG do not act on a common central glucoreceptor when eliciting eating. Dissociation of insulin and 2-DG eating has been observed after zona incerta lesions (Walsh and Grossman,1975) or after midbrain coronal knife cuts (unpub. obs.) indicating that section of pallidal axons to the midbrain may be the source of this phenomenon. (d) PG thirst deficits after LH lesions (or cuts) may be confounded with dysfunction in Na regulatory mechanisms. (e) Recovery of function after aphagia and adipsia appears to be more dependent on adjustment within NE rather than DA systems. This may be especially true for manipulations such the present knife cuts that spare diencephalic monoamines while depleting striatal DA. A previous report by Berger et al. (1971) has shown that recovery of eating after LH lesions may be facilitated by intracranial injection of NE.

Berger, B.D.,C.D. Wise, & L. Stein, Science,172,281-284, 1971. Morgane, P.J., Am. J. Physiol.,201, 420-428, 1961. Walsh, L.L., & S.P. Grossman, Physiol. & Beh.,15,481-485,1975.

407 EATING FOLLOWING CHOLINERGIC STIMULATION OF THE HYPOTHALAMUS. William T. Chance and John A. Rosecrans. Dept. Pharmacol., Med. Col. Va., Richmond, Va. 23298.

The literature concerning the neuropharmacology of eating indicates that food consumption is mediated by noradrenergic systems. We have previously reported (Midwest. Psych. Assn., 1973), however, that application of crystalline carbachol (CARB) to the perifornical hypothalamus (PFH) of food- and water-satiated rats reliably elicits eating as well as drinking. This eating emerges gradually, requiring 2-3 days (1 stimulation/day) to reach asymptote (2 g/hr). CARB also elicits eating at brain loci where norepinephrine (NORE) is without effect, but CARB-elicited eating is more potent at NORE-positive sites.

Contrary to a report by Wise (Physiol. & Behav., 9, 659, 72) this effect does not appear to be a rebound phenomenon (Chance & Lints, Amer. Psych. Assn., 1974), since restimulations with crystalline CARB at 20 min intervals did not inhibit eating. Following the first 2 daily CARB stimulations in this study, eating occurred in the last half of the 60 min period and was negatively correlated with drinking. Across the next 3 days of the study, however, eating was observed primarily in the first 30 min after the CARB stimulations and was significantly positively correlated with drinking. Furthermore, restimulation with CARB at 20 min intervals on day 2, during the period of negative correlation between eating and drinking, did not inhibit eating.

Eating is also observed following the injection of solutions (1 ul) of CARB into the PFH of rats. Significant eating and drinking were observed in satiated rats following doses of CARB as low as 0.5 nanomoles (nmol) and appropriate dose-response relationships were shown for doses through 4.0 nmol (Chance, Amer. Psych. Assn., 1975). Eating following the injection of CARB does not appear to depend upon interactions with adrenergic systems, since it was not blocked by pretreatments (5 min) with equimolar doses of alpha- (phentolamine) or beta- (propranolol) adrenergic antagonists. Although pretreatments with equimolar doses of the muscarinic-cholinergic antagonist, atropine, effectively obviated drinking, they had no effect on the eating response. Pretreatments with equimolar doses of the nicotinic-cholinergic antagonist, hexamethonium, had no effect on either behavior. Similarly, pretreatments with equimolar doses of the dopaminergic antagonist, haloperidol, did not affect eating or drinking following cholinergic stimulation of the PFH.

Carbachol not only stimulates the postsynaptic cholinergic receptor, but also elicits the release of endogenous acetylcholine (ACH). Effective release of ACH can also be accomplished by inhibiting its hydrolysis. Thus the injection (1 µl) of 16 nmol of the cholinesterase inhibitor, eserine, into the rat PFH was followed by increased food and water consumption. The CARB-elicited release of ACH should be blocked by pretreatment (1 hr) with the inhibitor of choline uptake, hemicholinium-3 (HC-3). The injection of 12 nmol (1 µl) of HC-3 into the PFH of cannulated rats 1 hr before CARB stimulation (4 nmol) reduced the eating response by 81% and drinking response by 24% of those receiving saline (1 µl) 1 hr before the CARB stimulation. Eating following the injection of eserine was also obviated by pretreatments with HC-3.

Thus although the eating response following CARB stimulation of the PFH does not appear to result from the muscarinic or nicotinic actions of ACH, or interactions with the adrenergic or dopaminergic systems, it does require the endogenous release of ACH. This eating response also seems to be a direct effect of this released ACH and not a phenomenon of disinhibition.

408 PREGASTRIC STIMULI ALONE DO NOT ELICIT NORMAL MEAL SIZE IN RHESUS MONKEYS. John D. Falasco*, James Gibbs, and Gerard P. Smith. Dept. of Psychiatry, Cornell University Medical College and E.W. Bourne Behavioral Research Laboratory, The New York Hospital-Cornell Medical Center, White Plains, N.Y. 10605.

In order to identify the anatomical site of origin of the unknown satiety signals released when food is eaten, we equipped adult male rhesus monkeys with chronic gastric cannulas which could be temporarily opened to allow gravity drainage and recovery of an ingested liquid food. After recovery from surgery, the monkeys were adapted to chronic restraint in primate chairs housed in individual booths, to a 12-hr light-dark cycle (0700-1900 lights on), and to the following feeding schedule: Overnight food deprivation, 1730-1000; access to liquid food (chocolate-flavored Nutrament, Drackett, 1 Kcal/ml) during a 90-minute test period, 1000-1130; and access to solid food (Purina monkey chow pellets), 1130-1730. Tap water for drinking was available at all times. Throughout the test period, 3 observations were recorded at 3-min intervals: Liquid food consumption, recovery of gastric drainage, and behaviors of individual monkeys. On days when gastric cannulas were closed, we found that each monkey appeared to sleep (was observed to be motionless with eyes closed) soon after an initial bout of intensive feeding; we used this behavioral criterion of apparent sleep to define the end of a meal. Results: In the last closed-cannula test after intake had stabilized, 5 monkeys had a first meal size of 392 ± 84 ml. ($\bar{X}$±SE) and the duration of this first meal was 23 ± 9 min. Every monkey appeared to sleep within 21 min of the end of the meal, and the duration of apparent sleep was 26 ± 10 min. On the next day, when gastric cannulas were opened for the first time, every monkey sham-fed a larger amount (941 ± 268 ml, $p < .05$, compared to the previous day's closed condition) and sham-fed longer (52 ± 14 min, $p < .05$). However, 4 of 5 monkeys did stop sham-feeding at some point during this first open-cannula test. Duration of apparent sleep was only momentary when it was observed to occur at all (1 ± 1 min, $p < .025$). On 5 sequential test days when gastric cannulas were opened in 4 monkeys, the amount sham-fed and the duration of sham-feeding increased steadily from 998 ± 280 ml in 53 ± 15 min (day 1) to 2788 ± 125 ml in 85 ± 9 min (day 5, $p < .005$); this increase over time progressed further until all monkeys sham-fed virtually continuously throughout the 90-min test period.

Summary: (1) Pregastric stimuli alone cannot elicit normal test meal size, (2) unusually prolonged pregastric stimulation can inhibit sham-feeding, and (3) this inhibition of sham-feeding by prolonged pregastric stimulation is fragile and extinguishes quickly over time.

Conclusion: Under these experimental conditions of diet and deprivation, normal meal size in rhesus monkeys depends crucially on the action of an unidentified satiety signal from the stomach or small intestine. (Supported by PHS Grant AM 17240).

409 PAIN ELICITED INGESTIVE SEQUENCES FROM CHRONIC DECEREBRATE RATS. Harvey J. Grill and Ralph Norgren. Rockefeller Univ., New York, NY 10021.

Central electrical and chemical stimulation will elicit organized behavior patterns that share many characteristics with regulated ingestive behavior. Peripheral nociceptive stimulation (tail pinch), a seemingly inappropriate stimulus, also elicits organized components of ingestive consummatory behavior. It has been suggested that the ingestive behavior elicited by tail pinch is dependent upon an intact nigrostriatal dopamine system (Antelman & Szechtman, 1975). Supracollicular decerebration, performed in two stages, eliminates all ascending connections of the nigrostriatal dopamine system. No descending connections from substantia nigra have been described. These chronic decerebrate preparations never spontaneously eat or drink. Nevertheless, tail pinch strong enough to elicit directed attack, also reliably elicited rhythmic lapping and chewing, but without regard to the presence of appropriate food objects. In a number of videotaped observations, rhythmic lapping was accompanied by sequential opening and closing of the forepaws. Unlike intact rats, the elicited consummatory behavior of chronic decerebrates frequently outlasted the nociceptive stimulus by at least 30 sec. While the forebrain may serve to organize and sequence ingestive behavior in the presence of appropriate goal objects the isolated caudal brainstem, void of all forebrain controls including the nigrostriatal dopamine system is capable of producing the components of ingestive consummatory behavior following a nociceptive stimulation. This fact suggests several alternatives for how the intact system may function during behavior elicited by painful peripheral stimulation. Upon stimulation, the forebrain may inhibit production of ingestive responses until suitable food or other goal objects are located. Conversely, the forebrain may direct food search only as a consequence of feedback from consummatory acts executed via the caudal brainstem. The fact that the elicited behavior does not extend beyond the period of peripheral stimulation in the intact rat, suggests that forebrain mechanisms are inhibiting the execution of brainstem consummatory behavior both during and following peripheral nociceptive stimulation. (Supported by NIH 5 F22 AM02360-02, NS 10150 and GM 1789.)

410 CHRONIC HYPERACTIVITY WITHOUT ADIPSIA OR APHAGIA INDUCED BY DISCRETE LESIONS OF PARS COMPACTA OF THE SUBSTANTIA NIGRA IN RATS. Gordon K. Hodge[1] and Larry L. Butcher[2]. Department of Psychology (1,2) and Brain Research Institute (2), University of California, Los Angeles, CA 90024, U.S.A.

Considerable experimental evidence indicating the involvement of the substantia nigra in the "lateral hypothalamic syndrome" comes from studies (Fibiger *et al.*,1973, *Brain Res.* 55, 135; Stricker and Zigmond, 1974, *J. Comp. Physiol. Psych.* 86, 973; Marshall *et al.*, 1974, *J. Comp. Physiol. Psych.* 87, 808) which utilized 6-hydroxydopamine (6-OHDA) to destroy the nigra. Some debate has arisen, however, over the degree of selectivity of this neurotoxin (Butcher, 1975, *J. Neural Trans.* 37, 189; Javoy, 1975, *J. Neural Trans.* 37, 219), and the possiblility that the non-selective effects of the drug might confound any interpretations based on its presumed selectivity must be entertained seriously (Butcher, 1975, *op. cit.*; Butcher *et al.*, 1975, in: *Chem. Tools in Monoamine Res., v. 1*, Jonsson *et al.*, eds., Amsterdam: North-Holland, p. 81; Stricker *et al.* and Zeigler and Karten, 1975, *Science* 190, 694).

Several previous experiments investigating the role of the nigra in eating and drinking behaviors have used doses and volumes of 6-OHDA, 8-10 µg/4 µl, which are larger than the dose, 2 µg/1 µl, found by Javoy *et al.* (1976, *Brain Res.* 102, 201) to result in selective damage to catecholaminergic neuronal somata and their processes. Nevertheless, Marshall *et al.* (1974, *op. cit.*), who used a relatively large dose, 8 µg/4 µl, have reported little non-selective damage in at least one case; others (see Butcher, 1975, *op. cit.*) have observed varying degrees of non-selective destruction following similar doses of 6-OHDA. Considering the variable degrees of selectivity reported with use of 6-OHDA, it is therefore difficult to evaluate adequately the significance of previous findings.

Comparisons among behaviors following administration of 6-OHDA and those, for example, after electrolytic lesions have been hindered by technical difficulties encountered in the use of the latter method in destroying sufficiently large amounts of pars compacta without concomitantly impinging on surrounding areas. By performining bilateral, oblique, double-insertion radio-frequency lesions of pars compacta, however, we have been successful in destroying at least 95% of the compacta without damaging pars reticulata or the medial lemniscus.

Such radio-frequency lesioned rats remained hyperactive, as measured in photocell activity cages, as long as 3 months after the lesions were made; some animals gave no indication of returning to pre-operative baseline levels at the time of sacrifice. The degree, and especially the duration, of hyperactivity appeared to correlate well with the extent of damage to pars compacta — i.e., the greater the destruction, the more intense and persistent the hyperactivity. In some respects, the nature of the hyperactivity resembled some features commonly associated with amphetamine-induced hyperactivity (e.g., gnawing of the cage bars, increased exploratory behaviors).

Although some hypophagia and hypodipsia were typically observed following surgery, food and water intakes were normal within 6-7 days. Forced food and water maintenance was never necessary. Creese and Iversen (1975, *Brain Res.* 83, 419) have also failed to observe adipsia and aphagia after 6-OHDA lesions of the substantia nigra despite as much as 99% depletion of dopamine levels in the striatum.

We cannot exclude the possibility that our lesions damaged fibers of passage through pars compacta which might have been "spared" by the use of appropriate doses of 6-OHDA (presumably 4 µg/2 µl or less; Bloom, 1975, *J. Neural Trans.* 37, 183; Javoy, 1975, *op. cit.*). We can agree, however, with other researchers (e.g., Fibiger *et al.*, 1973, *op. cit.*; Stricker and Zigmond, 1976, in: *Hunger*, Novin *et al.*, eds., N.Y.: Raven, p. 19) that it is unlikely that pars compacta *per se* plays an exclusive role in the mediation of food and water regulatory mechanisms. (Support: USPHS NS-10928 and Scott Fund)

411 OPERANT SCHEDULES DISSOCIATE THE REINFORCING VALUES OF LATERAL HYPOTHALAMIC SELF STIMULATION AND FOOD. Steven R. Hursh* and Benjamin H. Natelson. Dept. Exp. Psychology, Walter Reed Army Institute of Research, Washington, DC 20012; VA Hospital and Dept. Neuroscience, New Jersey Medical School, East Orange, NJ 07018.

A current controversy in neuroscience is whether the reward of lateral hypothalamic self stimulation (LHSS) is the same as the reward in eating, and thus by inference whether both reinforcers have the same neural stratum. One way to approach this problem is to measure preference for LHSS in comparison to food; if the reinforcers are functionally identical, then preference between them should be invariant with uniform changes in the environment. For example, if the availability of both types of reinforcers were parametrically restricted by operant schedules, producing, on the average, temporally equal access to each reinforcer, the value of LHSS should be as great as that for food under conditions of scarcity as under conditions of plenty.

We studied three rats 24 hr a day for nine months. Two rats had bipolar, platinum electrodes implanted in lateral tuberal hypothalamus and one in posterolateral hypothalamus. Rats always had equal access to both 0.5 sec trains of LHSS (0.2 msec, biphasic square wave pulses at 100 cps with current fixed at levels consistently producing high rates of responding on a CRF schedule), and 45 mg food pellets; delivery of both reinforcers was always preceded by a 0.5 sec light. Two equal but independent variable-interval (VI) schedules controlled the time between available reinforcers. Decreasing the rate of availability of both types of reinforcers in five steps from an average of one every 3 sec to one every 60 sec increased the rate of responding for food but decreased the rate of responding for LHSS at both sites. The rats changed their distribution of responses from a strong preference for LHSS at VI 3 sec to a strong preference for food at VI 60 sec. Table 1 shows an example of this shift in 24 hr responding for one of the subjects with a lateral tuberal placement. As a result of this shift in responding, the rats received a relatively constant number of food pellets per day across conditions but a monotonically decreasing number of LHSS trains per day (see table).

This dissociation between the value of LHSS and food as reinforcers was confirmed in a second experiment with current intensity 2.5 times higher. Although LHSS response rates were consistently elevated compared to the first experiment, preference still shifted toward food with longer times between reinforcers.

These data indicate that the value of LHSS as a reinforcer relative to food can be greatly reduced by environmental constraints on availability even when highly preferred under conditions of frequent availability. Since the reinforcement schedules for both types of reinforcement were always identical and since no changes in food consumption were observed, these results demonstrate that LHSS is not functionally equivalent to food. The dissociation in the reinforcing value of LHSS and food is evidence that the neural systems in lateral hypothalamus for each of these reinforcers are not identical. (Supported in part by VA grant #5983-02 to BHN).

Table 1

VI Schedules	LHSS		FOOD	
	Resp/hr	Reinf/hr	Resp/hr	Reinf/hr
3 sec	118	49	17	15
15 sec	28	6	67	18
60 sec	13	3	138	14

412 PREABSORPTIVE STIMULI ALONE ARE SUFFICIENT FOR NORMAL MEAL SIZE AND INTERMEAL INTERVAL. F. Scott Kraly, William J. Carty*and Gerard P. Smith. Dept. of Psychiatry, Cornell U. Med. Coll. and E.W. Bourne Behavioral Research Laboratory, New York Hospital, White Plains, N.Y. 10605

During sham-feeding ingested food drains out an open gastric fistula (GF), does not accumulate in the stomach, and cannot enter the small intestine. After 3-hr food deprivation, rats sham-fed a larger first meal ($p < .05$) and returned to eat a second meal sooner (Intermeal Interval, $p < .02$) than when they fed with the GF closed (see Table, Closed Fistula vs. Sham-Feeding). After each sham-fed meal, however, rats did exhibit a behavioral satiety sequence of grooming and exploring culminating in resting with a normal latency ($p > .10$). Thus, if normal satiety is defined as normal meal size (MS) followed by a specific behavioral sequence culminating in resting or sleeping and a normal intermeal interval (II), then pregastric (oropharyngeal) stimulation alone (i.e. sham-feeding) is not sufficient for normal satiety in rats, although pregastric food-contingent stimuli are sufficient for display of the behavioral sequence of satiety.

We examined the ability of the gut hormone cholecystokinin (CCK), a putative preabsorptive satiety signal, to normalize MS and II in sham-feeding rats. 8 rats were tested in 3 conditions following 3-hr food deprivation: (1) normal feeding (GF closed); (2) sham-feeding (GF open) with 0.9% NaCl i.p.; (3) sham-feeding with CCK i.p. (30 U/kg body weight; 20% pure, GIH Research Unit). All injections were given 1 min prior to the feeding test.

	Closed Fistula	Sham-Feeding	Sham-Feeding & CCK
First Meal Size (ml)	5.0 ± 0.5	16.1 ± 3.8	5.4 ± 2.0
Latency to Rest (min)	10.8 ± 1.2	18.8 ± 4.5	9.6 ± 1.9
Intermeal Interval (min)	91.5 ± 21.8	29.3 ± 10.0	80.8 ± 14.6

($X \pm SE$; $N = 8$; Latency to Rest = time from initiation of feeding to first instance of resting or sleeping)

Injection of CCK prior to sham-feeding normalized MS and II: (1) MS during sham-feeding with CCK was the same ($p > .20$) as MS with GF closed and less ($p < .05$) than MS during sham-feeding without CCK. (2) II following the first sham-fed meal with CCK was the same ($p > .10$) as II when feeding with GF closed and longer ($p < .05$) than II following a sham-fed meal without CCK. Latency to rest following a meal did not differ ($p > .10$) among treatments. These results show that, under our experimental conditions, preabsorptive stimuli alone (pregastric and CCK) are sufficient to produce normal satiety because they produce the same MS, the same behavioral sequence of satiety and the same II as we measured in these rats when they fed with the GF closed. (Supported by Lineberry Fund and NIH grants.)

413 FEEDING ELICITED BY NORADRENERGIC STIMULATION OF THE PARAVENTRICULAR NUCLEUS: EFFECTS OF CORTICOSTERONE AND OTHER HORMONE MANIPULATIONS.
Sarah Fryer Leibowitz, Kevin Chang*, and Robert L. Oppenheimer*.
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Our earlier studies on noradrenergic control of feeding behavior in the rat have demonstrated that: 1) the hypothalamic paraventricular nucleus (PVN) is the most responsive site with respect to the elicitation of feeding by central norepinephrine (NE) injection, and 2) this NE-elicited feeding response is essentially abolished by hypophysectomy. Further studies into the dependence of this PVN feeding phenomenon on the pituitary have provided evidence to suggest that hormones controlled by the pituitary may play a permissive role in the maintenance of normal PVN noradrenergic function. Moreover, the hormones mediating this effect appear to arise, at least in part, from the adrenal glands, with the glucocorticoid, corticosterone, being one of the key hormones involved.

Our evidence includes the following. 1) As with hypophysectomy, adrenalectomy was found to greatly diminish the rats' responsiveness to PVN noradrenergic stimulation. The eating response of 2.8 g (of lab chow) induced by NE injection in intact satiated rats (tested in the morning) decreased to 0.8 g in adrenalectomized rats and 0.2 g in hypophysectomized rats. Thyroidectomized, gonadectomized, and sham-adrenalectomized rats exhibited normal or even enhanced responsiveness to NE. All rats tested, including adrenalectomized and hypophysectomized animals, responded normally to the stimulating effects of other drugs (such as carbachol, insulin, and histamine) on ingestive behavior. 2) In an attempt to restore the sensitivity of hypophysectomized rats to PVN noradrenergic stimulation, an initial experiment was conducted in which four key hormones were replaced simultaneously. These hormones, which were injected daily (in the afternoon) over a period of approximately 14 days, consisted of insulin and the three target organ hormones, l-thyroxine, testosterone, and corticosterone. This combination of hormones was found to significantly restore (to 1.7 g) the responsiveness of hypophysectomized rats to central NE stimulation. This restoration appeared to be due primarily to corticosterone, since removal of this hormone from the daily replacement therapy returned the rats' NE behavioral response to the 0.3 g level. 3) Experiments in which the above four hormones were administered separately into hypophysectomized rats showed that l-thyroxine, testosterone, or insulin had little effect on the rats' sensitivity to NE. Corticosterone replacement, however, revealed a significant NE feeding response of 1.1 g. This response increased to 1.8 g with replacement of insulin in combination with corticosterone, and strengthened slightly more, to 2.1 g, with added replacement of deoxycorticosterone. This mineralocorticoid hormone, when administered with insulin but in the absence of corticosterone, revealed a slight NE response of 0.7 g. 4) Consistent with the results obtained in hypophysectomized rats, experiments with hormone replacement in thyroidectomized and gonadectomized rats showed little effect of l-thyroxine and testosterone on the rats' responsiveness to PVN injection of NE. Corticosterone, in contrast, was found to significantly restore the NE feeding response which was greatly attenuated in adrenalectomized rats. In the presence of this hormone, these rats exhibited a reliable feeding response of 2.0 g, as opposed to 0.8 g without corticosterone replacement. In preliminary studies, the effectiveness of this glucocorticoid hormone in restoring PVN noradrenergic function in adrenalectomized rats did not appear to be shared by the mineralocorticoid, deoxycorticosterone.

These results may be related to the findings of other studies which show normal feeding periods to be preceded by circadian rises in endogenous corticosterone.

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414 PARADOXICAL POSITIVE FEEDBACK EFFECT OF LARGE DUODENAL OR INTRAPERITONEAL LOADS OF GLUCOSE, FRUCTOSE AND MANNOSE ON SUBSEQUENT FOOD INTAKE. M. Rezek*, V. Havlicek, R. Jell and K.R. Hughes*. Dept. Physiol., Med. College, Univ. of Manitoba, Winnipeg, Man. R3E 0W3, Canada.

We have recently reported (Rezek, Havlicek & Novin, Am. J. Physiol., 229, 545-548, 1975) that large duodenal loads of isotonic glucose (30 ml, 1 ml/min) reverse the satiating effect of smaller glucose loads which is mediated by the afferent vagus (Rezek and Novin, J. Nutrit., in press) and paradoxically stimulate food intake (FI). While underlying mechanisms are presently being investigated it is also of interest to determine how this phenomenon is affected by a variety of experimental and nutritional conditions. Thus a recent series of experiments has revealed that paradoxical stimulation of FI can also be induced by the glucose analogues - fructose and mannose with fructose being most effective in increasing FI (33% in 1st hr) and shortening the latency of the first postinfusion meal (by 17 min). Total daily food consumption was not affected as no compensatory reduction toward the maintenance of caloric balance was observed. Stimulation of FI was also observed after both duodenal and intrapenitoneal infusions of larger volumes of glucose in free-feeding and 12 hr food-deprived animals. A similar paradoxically increased feeding response was induced by repeated daily duodenal infusions of larger glucose loads which, in addition, gradually increased total daily food intakes. Water-deprived animals (12 hr) allowed to drink glucose (5%) for 1 hr and water for the remaining 11 hrs drank 94 ml, thus providing 59% of their calories of carbohydrate origin supplied in control animals (drinking water for 12 hr) by regular food. Despite this substantial caloric preloading, FI during the 1st hr (when animals were allowed to drink glucose) was practically the same as the intake of control animals, thus resulting in a significantly larger intake of calories of carbohydrate origin. This caloric imbalance persisted during the subsequent 2 hr but tended to decrease over a 24 hr period. These data are consistent with the hypothesis that the prolonged larger loads of glucose and its analogs introduced via two different routes and nutritional conditions may stimulate insulin release not only from the acutely releasable functional pool but also from slowly reacting chronic pools. This would result in a preferential removal of the circulating energy substrates via the insulin-dependent lipogenic pathways at the expense of cellular energy supply, thus constituting a stimulus for the paradoxical stimulation of food intake.

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415 COMPARISON OF ACTIVITY OF NEURONES IN THE INFEROTEMPORAL CORTEX AND REGION OF THE SUBSTANTIA NIGRA WITH THE ACTIVITY OF NEURONES IN THE LATERAL HYPOTHALAMUS DURING FEEDING. E.T. Rolls, M.J. Burton*, S.J. Judge*, G.J. Mogenson, F. Mora* and M. Sanghera*, Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, Great Britain.

Neurones in the monkey lateral hypothalamus and substantia innominata alter their activity prior to and/or during feeding, when for example the hungry monkey looks at food (Rolls et al., 1976; Burton et al., 1976). To investigate the extent to which these responses might be different from sensory or motor responses, recordings were made in the monkey in the same test situation from neurones in the inferotemporal visual cortex, and the region of the substantia nigra.

In recordings made in the inferotemporal cortex during feeding, it was found that some neurones responded selectively to different visual stimuli with latencies of 140-230ms, and that the responsiveness of these neurones was not affected by whether or not the stimulus was associated with food, or by the hunger of the animal. Thus at this stage of information processing, learning about food stimuli, and satiety, did not affect the responsiveness of neurones.

When recordings were made from neurones in the region of the substantia nigra or the globus pallidus, some neurones were found which fired during feeding, in relation to motor movements such as mouth movement, and reaching. These responses were thus different from the activity of at least some lateral hypothalamic neurones, which may respond after the animal sees food, but before and not in close relationship to the mouth or arm movements in which some nigral and pallidal neurones were active during feeding.

These findings provide information on the processes taking place in different brain areas during feeding, and suggest that some neurones in the lateral hypothalamus and substantia innominata have a function which is different from that of motor or sensory function, and that they could be involved in some aspect of feeding such as the autonomic, endocrine or eating responses which occur in the hungry animal to food (Rolls, 1975, 1976).

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416 SEXUAL DIMORPHISM IN THE RESPONSE TO DIFFERENT DIETS. J. K. Young*, D. M. Nance and R. A. Gorski. Dept. Anat. and Brain Res. Inst., Sch. Med., UCLA, Los Angeles, CA. 90024. (SPON: Bruce Bromley).

The relation between androgen dependent sexual differentiation of the brain and the regulation of caloric intake (CI), body weight (Bwt) and sensitivity to estrogen has been examined. Adult male, female and androgenized female rats (AF-1 mg testosterone propionate on day 1 of life) were divided into 3 groups and given ad lib access to 3 different diets: 1) powdered rat chow (C); 2) high carbohydrate diet (D)--2 parts chow:1 part dextrose; and 3) high fat diet (F)--2 parts chow:1 part Crisco. CI and Bwt were measured daily for 22 days prior to gonadectomy, and the responses to gonadectomy and to 6 μg estradiol benzoate (EB) 22 days later were measured.

Female rats initially overate for 3 days on the F diet but quickly adjusted their daily CI to the same level as C-fed females. In contrast, CI and Bwt for D-fed females were chronically lower (18% lower for CI) than that of C-fed and F-fed females throughout the experiment. This undereating on the D diet was not due to aversive taste properties of D, since we determined that females show a 93% preference for D over C diets when given a preference test. All female groups increased CI and Bwt after ovariectomy and both measures decreased in response to EB, with the F-fed females showing the largest decrease. On the other hand, males chronically overate and weighed more on the F diet relative to C-fed males, but unlike females, did not undereat on the D diet. After castration, CI decreased only for the C-fed males. Males were less responsive to EB than the females: maximal CI depressions from baseline after EB were 17% for males and 36% for females. AF rats, like males, overate on the F diet for the first 10 days, but then decreased CI to levels comparable to C-fed AF animals. AF rats also differed from females in that they did not undereat on the D diet. CI was unaltered by ovariectomy in AF rats and, like males, showed an attenuated response to EB.

The response to gonadectomy for these animals is presented in the table below.

Mean (± S.E.) Total Caloric Intake Over 19-day Periods

		Pre-gonadectomy	Post-gonadectomy
Females	Chows	1278.6 ± 38.7	1422.4 ± 94.4
	Dextrose	1046.3 ± 24.4	1184.4 ± 92.5
	Fat	1252.6 ± 37.2	1502.8 ± 71.9
Males	Chows	1651.9 ± 51.0	1542.2 ± 32.6
	Dextrose	1589.8 ± 39.2	1536.9 ± 36.7
	Fat	1974.5 ± 70.9	1908.5 ± 111.0
AF	Chows	1406.1 ± 37.7	1497.1 ± 77.2
	Dextrose	1309.2 ± 76.9	1327.7 ± 80.1
	Fat	1460.9 ± 74.0	1471.0 ± 101.8

The present data are consistent with the hypothesis (P.B.& B. 3: Suppl. 1, 155-162, 1975) that the process of perinatal androgenization permanently reduces the precision of long-term regulation of body energy balance. In addition, results found with the D diet indicate a heightened long-term satiating potency of this diet independent of its high palatability, supporting a glucostatic concept of long-term control of feeding behavior. (Supported by USPHS Grant No. AM-18254-01.)

417 WAVE FORM DURATION AND STIMULUS-BOUND BEHAVIOR IN THE RAT. Michael J. Ackerman. Behavioral Sciences Department, Naval Medical Research Institute, Bethesda, Maryland 20014.

Behavioral observations were made on rats bearing electrodes chronically implanted in the lateral hypothalamic area (LHA). The animals were tested for stimulus-bound eating (SBE) or drinking (SBD) behavior using a 30 sec train of 60 Hz current and the results noted. The animals were then retested using 1/4, 1/2, 1, 2, 4, and 8 sec pulse of 60 Hz current at a 50% duty cycle for 30 seconds. SBD could be elicited at a lower pulse duration from animals previously displaying SBD behavior than could SBE from animals previously displaying SBE behavior. This result is consistent with the notion that the drive level for thirst increases faster than the drive level for hunger.

418 REVERSIBLE HYPERPHAGIA AND OBESITY FOLLOWING INTRACEREBRAL MICROINJECTIONS OF COLCHICINE INTO THE VENTROMEDIAL HYPOTHALAMUS OF THE RAT. D. Avrith* and G. J. Mogenson, University of Western Ontario, London, Canada.

It has been shown that colchicine reversibly depresses synaptic transmission by blocking intraaxonal transport in treated neurons (Perisic and Cuenod, Science 175: 1140, 1972). The present study has attempted to produce a reversible blockade of the ventromedial hypothalamic nucleus (VMH) where bilateral destruction typically results in hyperphagia and obesity. Male Sprague-Dawley rats were injected bilaterally into the VMH with either 1 µl of isotonic saline or 1 µl of isotonic saline containing 2 µg of colchicine. After 48 hr the colchicine-treated rats began to eat voraciously and continued to do so for 5 days. During this period, food and water intakes as well as daily body weight gain were 40-50% greater than controls. Thereafter, the colchicine-treated rats reduced daily food intake until their body weight had returned to control levels. The temporary reduction in food intake may reflect a regulatory response to decrease excess body weight to a normal level rather than a nonspecific effect of colchicine. Colchicine-treated rats, which were restricted to the food intake of the controls for one week following injection, showed no compensatory reduction in food intake when later permitted free access to food and continued to maintain normal body weight gain. The results indicate that a temporary colchicine-induced blockade of neural activity in the VMH can lead to a reversible hyperphagia and suggest that colchicine may be useful in producing a reversible blockade in other discrete neural systems.

(Supported by the National Research Council of Canada).

419 EFFECT OF GASTRIC WATER LOADING ON UNIT ACTIVITY OF THE LATERAL HYPOTHALAMUS AND LATERAL PREOPTIC AREA. F. C. Barone*, M. J. Wayner, C. S. Weiss and C. R. Almli. Brain Res. Lab., Syracuse University, Syracuse, NY 13210 and Dept. Psychol., Ohio University, Athens, OH 45701. (SPON: B. Beer).

The effect of slow intragastric infusions of water on unit activity of cells in the lateral hypothalamus (LH) and lateral preoptic area (LPA) was determined. Both 24 hr water deprived and ad lib female rats were used for study. Following a 5 min baseline of stable unit activity water was intubated directly into the stomach at a rate of 1.15 ml/min over a 10 min period. A subsequent post infusion period of 5 min was also recorded and analyzed for all cells. A gastric catheter which allowed distension alone and/or application of water to the stomach was utilized under the same conditions to determine the relative effects due to stomach distension and water absorption. Results indicate that neurons of the LH and LPA are responsive to sensory inputs associated with intragastric administration of water. Lateral hypothalamic cells changed discharge frequency with relatively shorter latencies than LPA cells studied under the same conditions. Long term changes in the discharge frequency of these cells are complex and because they depend on plasma osmolality changes resulting from absorption of water, are also related to prior deprivation.

420 RESIDUAL DEFICITS TO PERIPHERAL AND CENTRAL THIRST CHALLENGES AFTER PERIVENTRICULAR LESIONS OF THE ANTERO-VENTRAL THIRD VENTRICLE (AV3V). J. Buggy, A.K. Johnson, D. Bert*, B. Packwood*. Depts. Physiol. & Psychol., Univ. of Iowa, Iowa City, Iowa 52242.

Angiotensin must contact periventricular tissue of the AV3V to induce drinking after intraventricular injections (Buggy et al., Science, 190: 72, 1975); the AV3V is also highly sensitive to local hyperosmotic stimulation for elicitation of thirst (Buggy, Physiologist 18: 156, 1975). These data suggest that the AV3V plays a key role in the detection of stimuli associated with body fluid depletions. Electrolytic lesions of tissue around the AV3V result in adipsia (but not aphagia) and a rapid loss of large volumes of dilute urine. Fluid replacement therapy prevents death during this hydrational crisis and voluntary ad lib water intake eventually returns (Johnson and Buggy, Fed. Proc., 35: 814, 1976). In these 'recovered' rats, hypovolemia stimulates water intake but drinking responses to intracellular dehydration and to both peripheral and central injections of angiotensin are no longer present. Some AV3V lesions result in a residual drinking deficit to intracellular dehydration only, suggesting that angiotensin and hyperosmolarity act on separate and independent neural substrates. Since AV3V lesions eliminate responses to angiotensin delivered to brain from either CSF or blood, and since ventricular plugs which prevent hormone spread to the AV3V block the action of intraventricular injections of angiotensin without disrupting neural connections or transmission, we conclude that receptors for central angiotensin mechanisms are localized in periventricular tissue surrounding the AV3V. Supported by grants from the Iowa Medical Research Council, NIMH RSDA 1K02 MH00064-01, and NSF BNS 75-16346.

421 OSMOREGULATORY THIRST IN RATS FOLLOWING LATERAL PREOPTIC LESIONS.
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Rats prepared with lesions in the lateral preoptic area (LPO) did not drink during the first 4 hr following the administration of 2 M NaCl solution (2 ml, i.p.), whereas control rats drank 10-17 ml water. In contrast, many (but not all) lesioned rats drank normally when hypovolemia was induced by subcutaneous injection of polyethylene glycol solution. Identical findings have been reported previously (Blass and Epstein, JCPP, 76: 378-394, 1971) and interpreted as indicating that the LPO contains osmoreceptors for thirst in rats. However, when we extended our tests to 24 hr after the injection of hypertonic saline, we found that the lesioned animals did drink and thereby restored osmotic equilibrium. Furthermore, we found that lesioned rats increased their daily water intakes as much as control rats did when salt was added to the diet or when hypertonic saline was infused intravenously. Indeed, many animals which had not consumed any water in the 4-hr test when hypertonic saline was given intraperitoneally drank normally when the same solution was given intravenously. These data indicate that the rat with LPO lesions is capable of experiencing thirst following acute osmotic imbalances. Together with other findings, they suggest that LPO lesions do not specifically abolish osmoregulatory thirst but instead produce a general intolerance of stress that in many ways seems analogous to the impairments observed after lateral hypothalamic lesions (Stricker, JCPP, 90: 127-143, 1976). (Supported by NIMH Grant MH-25140.)

422 EFFECTS OF SINGLE VERSUS GROUP HOUSING ON BODYWEIGHT GAIN OF MALE RATS WITH UNILATERAL LESIONS OF LATERAL HYPOTHALAMUS. Donald V. Coscina, Section of Neurochemistry, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario, Canada M5T 1R8.

Lateral hypothalamic (LH) lesions seem to chronically lower the bodyweight (BW) "set-point" (SP) of male rats to some fixed level of normal (Powley & Keesey, J. comp. physiol. Psychol. 70:25, 1970). To the extent that the greater BW gain of single (S) versus group (G) housed (H) rats represents different conditions dictating SP control, I wondered if recovery from and subsequent BW gain after unilateral (U) LH lesions would be seen as some constant decrement of control levels regardless of H. To test this possibility, 60 male rats averaging 200 g BW were SH (n = 30, single cages) or GH (n = 30, 5 per double cage) for 5 weeks, after which 12 per condition received sham lesions and 18 per condition received left hemispheric ULH lesions (55° C for 1 min at AP 6.0, ML 2.0, DV -8.5, head flat). After surgery, half of each subgroup was returned to its original H while the other half was switched to the opposite H. For GH rats, 3 ULH and 2 shams comprised each G. BWs were recorded daily for the first post-operative week and weekly thereafter for 7 weeks. When measures of daily BW for the first week were expressed as either percentage of or g difference from controls, 3-way analyses of variance across all 4 ULH subgroups revealed significant ($ps < .001$) interaction effects between time and both pre- and post-operative H factors. Similar analyses of weekly data revealed only significant ($p < .05$) interaction between time and post-operative H on g difference but not percentage measures. These findings imply that (a) different SP mechanisms operate during versus after recovery from ULH injury, and (b) SP "theory", at least in this experimental model, has predictive value only when BW is expressed in relative (percentage of control) units.

423 EFFECTS OF ANOREXIC AGENTS ON MEAL PATTERNS IN GENETICALLY OBESE AND LEAN ZUCKER RATS. A. Drewnowski* and J.A. Grinker* (SPON: V. Luine)
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Most studies on effects of anorexic agents have used meal-fed paradigms (2 to 6-hr access to food per day) or have measured 24-hr food and water intakes in chronically injected animals (Bray and York, Am.J.Physiol., 223:176, 1972: Sofia and Barry, Psychopharm.,39:213, 1974: Drewnowski, WCBR, 1975). These methods fail to specify the temporal courses of action of different drugs and do not distinguish between alternative modes of action of anorexic agents. These studies have rarely employed any appropriate animal model of obesity.

In the present study, analysis of meal patterns was used to determine the temporal course of anorexia following intraperitoneal (IP) administration of d-amphetamine (1.5 and 3.0 mg/kg), fenfluramine (5.0 and 10.0 mg/kg) and Δ9-tetrahydrocannabinol (THC)(1.0, 4.0 and 8.0 mg/kg) in genetically obese and lean Zucker rats. The use of a drinkometer circuit connected to an on-line PDP8 computer allows continuous monitoring of feeding over 24 hours and precise measurements of food intake over short intervals. Concomitant measures of running wheel activity provide the necessary control for hyper-activity or general malaise. The technique allows specification of meal frequency, meal size, inter-meal intervals and meal and activity interactions (Becker and Kissileff, Am.J.Physiol.,226:383, 1974).

Obese animals were generally more resistant to the action of anorexic agents, in agreement with Bray and York (op. cit.). Temporal profiles for amphetamine, fenfluramine and THC action were different. These findings are in partial agreement with Blundell and Leshem (Recent Advances in Obesity Research I:368, 1975), suggesting that anorexic drugs may act specifically upon meal initiation (meal frequency) or upon satiety (meal size).

424 DIFFERENT RECEPTORS INITIATE ADRENAL SECRETION AND HUNGER DURING HYPOGLYCEMIA. M.I. Friedman*, N. Rowland*, C. Saller*, E.M. Stricker (SPON: R. Feldman). Depts. Psychol., Univ. of Mass., Amherst, MA. 01002 and Univ. of Pittsburgh, Pgh., PA.

Adrenal discharge of catecholamines (CA) and increased feeding behavior during hypoglycemia are believed to be triggered by central receptor cells which are activated by cytoglucopenia. Intravenous infusion of .6M dl-β-hydroxybutyrate (HB), a metabolic fuel which can be oxidized by brain, abolished adrenal CA secretion in conscious rats during hypoglycemia induced by insulin (3U/kg, iv), whereas infusion of 1.2M fructose, a sugar which does not cross the blood-brain barrier, did not. In contrast, increased feeding behavior during hypoglycemia was prevented by infusion of fructose but not by infusion of HB. Infusions of 1.2M mannose or 1.2M glucose, two hexoses which can be oxidized by brain and peripheral tissue, prevented both increased feeding and CA discharge after insulin injections. Hypoglycemia persisted in all infused rats except those given glucose which were normoglycemic. Control infusions of 1.2M NaCl did not prevent increased food intake after insulin, and infusions of fructose or HB without insulin produced, respectively, no change in plasma CA or food intake from untreated control levels. These results indicate that while the sympathetic response during hypoglycemia may be initiated by receptor cells in the brain, the feeding response evidently is not. Because the liver is the only organ that cannot oxidize ketone bodies, the failure of HB to attenuate feeding after insulin may indicate that the stimulus for hunger during hypoglycemia has a hepatic origin.

425 SUSTAINED POST-STIMULATION SUPPRESSION OF DRINKING FOLLOWING ELECTRICAL STIMULATION OF THE SEPTUM IN THE RAT. Frank J. Gordon and Alan Kim Johnson, Dept. of Psychology, Univ. of Iowa, Iowa City, IA 52242.

The septal area has been implicated in the neural control of thirst. Lesions of the septum result in polydipsia (Harvey & Hunt, J. Comp. Physiol. Psychol. 59: 49, 1965), while electrical stimulation during access to water suppresses drinking (Wishart & Mogenson, Physiol. and Beh. 5: 1399, 1970; Blass, Neuroscience Abstracts 1: 470, 1975). Early investigators (Delgado & Anand, Am. J. Physiol., 172: 162, 1953; Smith, p. 367, in D. E. Sheer (Ed.), Electrical Stimulation of the Brain, 1961) have shown a long lasting post-stimulation effect of electrical brain stimulation on food consumption. The present study was designed to investigate the effect of a discrete period of brain stimulation on the subsequent intake of water.

Rats were implanted with bipolar stimulating electrodes in the ventromedial septum and maintained on either a 23 hr food or water deprivation schedule. After intakes had stabilized, they were stimulated (30 sec stimulation on--30 sec off, 30-90 μA rms, 60 Hz AC, one 500 msec pulse/sec) for 1 hr prior to 1 hr access to food or water. Water intake was reliably suppressed (22.7 $\pm$ .89 (SEM) (stimulated) vs. 29.9 $\pm$ .98 (control), $n = 4$, $p < .001$) during the 1 hr post-stimulation period with no effect observed for solid food consumption (11.8 $\pm$ .99 vs. 11.1 $\pm$ .68). As a further control, additional rats were implanted with electrodes at the same stereotaxic coordinates and allowed 1 hr daily access to a liquid diet supplemented by 5 g of food pellets in the home cage. One hr of stimulation had no effect on the post-stimulation intake of the liquid diet.

These data demonstrate a sustained and specific suppression of deprivation induced drinking when water was made available following a discrete period of electrical stimulation of the septum and further implicate this neural structure in the control of drinking.

426 EFFECTS OF ANGIOTENSIN ON CELLS IN THE PREOPTIC AREA OF RATS. R. J. Gronan and D. H. York. Dept. of Physiology, Sch. Med., U. of Missouri, Columbia, Mo. 65201

The role of circumventricular structures in the mediation of angiotensin (AII) induced changes in water intake and blood pressure has remained unclear. The present study was undertaken to examine the effects of AII, acetylcholine (ACh) and other possible neuromodulators on cells of the periventricular nucleus of the preoptic area (AP7.3, LO.3, D6.3-7.7). Experiments were carried out on 4 male Sprague-Dawley rats anesthetized with urethane. Single unit activity was recorded through the center barrel of a five barrel micropipette. The other barrels contained DL-homocysteic acid, AII (Ileu^5-angiotensin), acetylcholine and GABA, which were applied iontophoretically on to single cells to assess changes in neural discharge frequency. The major effect of AII was to cause excitation. Of a total of 60 cells, 36 were excited, 6 depressed and 18 unaffected by AII. The excited cells were predominantly localized at a depth of 6.9-7.4 mm from the cortical surface. Furthermore, of 18 cells excited by AII in this region, 14 were also excited by ACh. Of 12 cells located in suprachiasmatic nucleus (depth 7.5-7.7 mm from cortex) 7 were excited by AII and 6 of these were also excited by ACh. Cells dorsal to these two areas (depth 6.3-6.8 mm from cortex) showed a more variable response to AII, although they were also predominantly excited by ACh (17 out of 24 cells). These results demonstrate an excitatory action of AII on cholinoceptive cells localized adjacent to the third ventricle in the preoptic area.

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427 SELF-STIMULATION AND FEEDING: LONG TERM DISINHIBITION OF BOTH BY VENTROMEDIAL HYPOTHALAMIC LESIONS. Luis Hernandez* and Bartley G. Hoebel. Dept. Psychol., Princeton Univ., Princeton, N.J. 08540

According to a well-known, but controversial theory, a lateral hypothalamic (LH) system involved in feeding is under tonic inhibitory control by a ventromedial hypothalamic (VMH) satiety system (1). Self-stimulation of the LH may involve neural reinforcement mechanisms for feeding. If so, VMH lesions which disinhibit feeding should also disinhibit LH self-stimulation. Earlier work showed LH self-stimulation was disinhibited immediately after VMH lesions (2). This was confirmed, but the self-stimulation effect was only transient even though hyperphagia persisted (3). We now report that lesions which cause hyperphagia and obesity can also cause a prolonged increase in lateral hypothalamic self-stimulation. Bilateral electrolytic lesions (1 mA anodal for 20 sec) were made with implanted, monopolar, platinum electrodes in the VMH region (A 6, L 0.5, D 8.5, level skull) of female Sherman rats that showed self-stimulation and stimulation-bound eating with an LH electrode (A 6, L 1.8, D 7.5). Food intake, water intake, body weight and 3 hrs of self-stimulation were monitored daily for 4 days before the lesions and 12 days (to date) afterwards. Five of six lesioned rats showed hyperphagia, polydipsia, body weight gain and increased self-stimulation (mean response/ 30 min.: prelesion, 593; postlesion, 921) lasting the full 12 days. Six sham lesioned controls showed no changes. We conclude that both feeding and LH self-stimulation are tonically inhibited by a VMH mechanism because both were enhanced for many days after VMH damage.

(1) Anand, B.D. and Brobeck, J. Yale J. Biol. Med., 1951, 24,123-140.
(2) Hoebel, B.G. and Teitelbaum, P.,Science, 1962, 135, 375-376.
(3) Ferguson, N. and Keesey, R. J. Comp. Physiol. Psychol., 1971, 74, 263-271.

428 EFFECTS OF LESIONS SURROUNDING THE ANTERO-VENTRAL THIRD VENTRICLE (AV3V) ON FLUID HOMEOSTASIS. A. K. Johnson, J. Buggy, and M. W. Houshk*. Dept. Psychol. and Dept. Physiol., Univ. of Iowa, Iowa City, IA 52242.

Periventricular tissue has been suggested as the site of action for stimuli which signal perturbations of body water (Johnson & Epstein,Brain Res. 86: 399, 1975). More specifically, access to regions surrounding the AV3V region has been shown to be critical for the dipsogenic action of CSF borne angiotensin II (Buggy, Fisher, Hoffman, Johnson, & Phillips, Sci. 190: 72, 1975), and injections of hypertonic solutions into the AV3V are highly effective as a means of eliciting drinking (Buggy, Physiologist 18: 156, 1975). In order to further evaluate the role of various portions of AV3V tissue in the control of water intake, midline stereotaxic lesions were placed in rats under ether anesthesia. Small bilaterally symmetrical lesions placed within the optic recess of the AV3V produced a profound adipsia without aphagia. In many cases the adipsia persisted until the animal died (usually 3 to 6 days following the lesion). If the adipsia was sustained,the animals eventually became anorexic. When appropriate hydrational therapy was imposed upon adipsic animals, they continued to feed and eventually (2 to 14 days post lesion) regained spontaneous drinking. Analysis of urine output showed that following surgery adipsic animals excreted a significantly greater volume of dilute urine as compared to animals that were water deprived following a sham lesion. This inappropriate loss of fluid served to exacerbate the dehydration produced by the adipsia, and as a result the plasma osmolarity of adipsic lesioned rats was significantly greater than that of sham lesion water deprived rats. These findings indicate that the integrity of periventricular tissue surrounding the AV3V is essential for the normal maintenance of fluid balance by both behavioral and internal control systems and is consistent with the interpretation that tissue surrounding this region contains receptors which mediate thirst. (NIMH RSDAII-1 K02 MH 00064-01; NSF-BNS 75-16346)

429 INCREASED EATING AFTER GABA BLOCKADE AT THE INTERPEDUNCULAR NUCLEUS OR SUBSTANTIA NIGRA OF RATS. J. Kelly*, G. Alheid*, and S.P. Grossman (SPON: E. Hess) Univ. Chicago, Chicago, Ill. 60637

Double walled cannulas were placed just dorsal to the substantia nigra (SN) or interpeduncular nucleus (IP) in male albino rats of the Sprague-Dawley strain. Animals were kept on a 12/12 light-dark cycle. Drug injections were given 4-5 hours after the onset of the light part of the cycle. Implantation of crystalline bicuculline resulted in increased eating in the six hours following the injection. Increased drinking was also observed, but less reliably. The best eating responses were obtained after GABA blockade in the IP. Implantation of crystalline picrotoxin resulted in amphetamine-like stereotypy in 3 of 12 animals.

These results suggest that descending striato-nigral GABA tract normally inhibits the SN and IP dopamine neurons. Disinhibition of eating by GABA blockade indicates that relatively specific destruction of GABA axons, with sparing of dopamine afferents to the caudate may contribute to the increased eating and hyperreactivity seen after lesions in the ventromedial hypothalamus.

430 AN ELABORATION ON THE ANTIDIPSOGENIC ACTION OF PROSTAGLANDIN E_1 (PGE_1). Nancy J. Kenney and Alan N. Epstein, Inst. Neurological Sciences, U. of PA, Phila., PA 19174

The effects of intracerebroventricularly administered PGE_1 on water intake induced by centrally administered carbachol, peripherally administered hypertonic saline, or water deprivation were studied in adult male rats. PGE_1, in doses of 1 µg or 100 ng per rat, significantly reduced water intake in all rats treated with 100 ng carbachol. Intakes were slightly, but not significantly, reduced when 10 ng PGE_1 was given in competition with carbachol. Water intakes induced by 20-hr water deprivation were reduced significantly by 1 µg PGE_1, and the depression remained evident for the entire first hour of the 4-hr test session. There was a slight, but insignificant, depression in water intake with 100 ng PGE_1. This decrease in intake was only evident for the first 20-min of the test session. Again, 10 ng PGE_1 had no effect on water intake. PGE_1 was not effective in modulating water intake stimulated by subcutaneous injection of hypertonic saline (0.75cc 2M NaCl/100g body weight) at any dose tested.

Previously, we have shown that intracerebroventricularly administered PGE_1 is a specific antidipsogen and is effective in reducing water intake stimulated by the central injection of angiotensin II in doses as low as 10 ng (Kenney & Epstein, Neuroscience Meeting, 1975; Epstein & Kenney, In Central Actions of Angiotensin and Related Hormones, Buckley & Ferrario (eds.), in press). It now appears that the effects of PGE_1 on water intake are specific to the type of challenge incurred and may offer a means by which the various mechanisms of thirst can be more closely analyzed.

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431 COMPARATIVE ASPECTS OF THE DEVELOPMENT OF GLUCOSTATIC MECHANISMS IN INFANT RATS AND GUINEA PIGS. Susan Dray Klauss, Dept. of Psychology, UCLA, Los Angeles, CA. 90024, Ellen G. Mooney*, Ann T. Ewing*, and Dennis A. VanderWeele*, Dept. of Psychology, Occidental College, Los Angeles, CA. 90041.

Infant rats and guinea pigs were tested throughout infancy to determine the age at which they first showed suppression of food intake following glucose infusions. While adult animals consistently reduce intake following infusions of glucose, the age at which this phenomenon develops has been the subject of recent controversy. Litters were removed from their mothers, kept for 3 hours without food or water, then intubated intragastrically with isotonic saline or 5% glucose. Three hours later, they were returned to their mother to suckle for 2 hours. Weight gain in this final 2 hour period was taken as a measure of ingestive activity. Rat pups (N=605) first responded to glucose intubation at 14 days, consistently suppressing intake by 30-50%. This suppression continued until days 20-21, at which time there was a transient period of lack of suppression, perhaps due to weaning. The advent of this phenomenon correlates closely with the maturation of liver enzymes important in glycogen deposition and glucose utilization. Previous results from our laboratory have demonstrated that these metabolic pathways in the liver are important in the control, via the vagus nerve (X), of food intake. Similar experiments with guinea pigs are currently in progress. Since guinea pigs show relatively advanced development at birth, possible species differences may be related to this precociousness. Results will be discussed as they relate to possible peripheral, liver glucoreceptor models.

432 THE OCTAPEPTIDE OF CHOLECYSTOKININ DECREASES LATERAL HYPOTHALAMIC SELF-STIMULATION. Carol L. Kornblith, G.N. Ervin*, and Richard A. King. Dept. Psych. and The Neurobiology Program, Univ. North Carolina, Chapel Hill, N.C. 27514.

Self-stimulation of the lateral hypothalamic area has been interpreted in terms of reward related to food. Manipulations which increase food intake increase lateral hypothalamic self-stimulation (LHSS), and those which decrease food intake decrease LHSS. We measured the effect of the C-terminal octapeptide of cholecystokinin (10-80 Ivy dog units/Kg) on LHSS because it decreases food intake and produces satiety behaviors without producing illness. We report that the octapeptide of cholecystokinin decreases LHSS in a dose-related fashion. These data suggest that the reward of LHSS may be related to food reward. However, we are currently looking at the effect of the octapeptide on self-stimulation at other brain sites less directly involved in feeding behavior.

433 EFFECTS OF CENTRAL LESIONS ON SCHEDULE DEPENDENT AND SCHEDULE INDUCED BEHAVIORS. C. C. Loullis*, M. J. Wayner and F. C. Barone*. Brain Res. Lab., Syracuse University, Syracuse, NY 13210. (SPON: R. Salafia)

Rats were reduced to 80% body weight and were exposed to an FI 1 min food reinforcement schedule for 30 min daily unit lever presses, licks, and water consumption stabilized for at least 10 days. Animals were then subjected to bilateral mid lateral hypothalamic (LH) lesions, bilateral posterior LH lesions or thalamic semilunar nucleus lesions. Testing continued for 50 days following the lesions at 80% body weight and for an additional 30 days during ad lib feeding and after body weight recovered. Lesioned animals were subjected to a series of tests designed to determine the extent of functional damage. The tests included: food consumption following food deprivation; fluid consumption following water deprivation; insulin induced eating; salt arousal of drinking; and temperature-taste fluid preference. On the basis of these tests and histological examination of lesion locus, the experimental animals were assigned to groups and were compared to sham lesion control animals. Results indicate that the neural mechanism which is involved in schedule induced polydipsia is destroyed by posterior bilateral LH lesions. The effects of the other lesions studied were evaluated and discussed in terms of LH involvement in schedule induced and schedule dependent behaviors.

434 NORADRENERGIC ACTIVITY IN THE HYPOTHALAMUS OF THE RAT IS ALTERED BY LOCAL GLUCOSE, INSULIN OR 2-DEOXY-D-GLUCOSE DELIVERED BY PUSH-PULL PERFUSION. M. L. McCaleb* and R. D. Myers. Depts. of Psychological and Biological Sciences, Purdue University, Lafayette, Indiana 47907

In rats on a daily feeding regimen, hypothalamic stores of norepinephrine (NE) were radiolabeled by 1.0 - 3.0 µCi of ^{3}H-NE micro-injected in a volume of 0.5-1.5 µl into the perifornical region. Successive push-pull perfusions of the site, at a rate of 25 µl/min, were undertaken so that a standard washout curve of radioactivity was generated. As the animal consumed food, ^{3}H-NE activity was augmented during the given interval of perfusion. When 2-deoxy-D-glucose (10 µg/µl) was added to the perfusion medium, a similar sort of enhancement was caused in ^{3}H-NE release. However, insulin (4.0 mU/µl) perfused similarly caused a delayed output of the catecholamine beyond the interval of perfusion. On the other hand, the addition of 5.5% glucose to the perfusate suppressed the hypothalamic efflux of ^{3}H-NE. The pattern of ^{3}H-NE release was dependent entirely upon the specific anatomical site of push-pull perfusion. These results suggest that a noradrenergic feeding system in the diencephalon not only mediates feeding behavior itself, but is involved also in the local monitoring of nutrient levels.

435 CONCURRENT MEASURES OF RENIN AND DRINKING IN RESPONSE TO HYPOVOLEMIA. R. Miselis, S. Nicolaidis*, M. Menard*, and Y. Siatitsas*. Sch. Vet. Med. Univ. Penn., Philadelphia, Pa. and College de France, Paris, France.

A group of 18 male rats(300-400g) prepared with chronic i.v. catheters for blood sampling were tested for drinking to hypovolemia produced by s.c. polyethylene glycol(PEG)(20%, 2ml/100g b.wt.). During these tests blood samples were taken for renin assay. Blood was removed remotely without disturbance to the rats' spontaneous behavior. Each rat was used twice, once for a control isotonic saline injection and once for a PEG injection with a one week interval. At the moment the rats went to take their first draft of water after the PEG injection their renin levels were significantly elevated above their pre-injection control levels(52.8 vs. 25.8). The renin activities after the control injection, when examined at approximately the same latency(42 min) to the first draft of water found on the PEG injection day, were not significantly elevated(40.2 vs. 28.0). The water bottles were removed at the initiation of drinking and drinking was prevented until 5 hours after the injections. At 5 hours renin activity had risen to 176 after PEG and remained within control values(23.7) after the control injection. Nine PEG injected rats, given isotonic saline to drink, drank 9.3ml in a 50 min drinking period following the 5th hour. Their renin values at the end of the drinking period were the same as at the beginning. Four PEG injected rats, given water to drink, drank 11.3ml in the same drinking period. Their renin values continued to rise(117 to 161). In experimentally produced hypovolemia renin levels are elevated at the onset of drinking. Whether this is causal or permissive is not known. Drinking water does not arrest the rise in renin. Drinking saline does.

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436 DEGENERATION IN PATHWAYS TO THE PONTINE TASTE AREA IN RELATION TO APHAGIA AND ADIPSIA. Elliott Mufson*, Saul Balagura and Walter Riss. Biol. Psychol. Program, Dept. Neurosurgery and Dept. Anat., SUNY Downstate Med. Ctr., Brooklyn, N.Y. 11203.

A striking finding emerged while assessing the anatomical degeneration pattern associated with aphagia and adipsia. After lateral hypothalamic lesions a much greater density of argyrophilic particles is found in the pontine taste area (PTA) in aphagic and adipsic rats than in non-aphagic rats with lesions. The PTA is a parabrachial nuclear zone which receives gustatory information from the nucleus of the tractus solitarius (Norgren and Leonard, 1973) and contributes to a pathway found in the extreme lateral hypothalamus (Norgren, 1976). We found a substantial pathway in rats of both sexes into the pontine taste area. Bilateral electrolytic lesions made in the extreme lateral hypothalamus with lesion parameters of 1 ma. for 7 or 10 seconds produced aphagia and adipsia and heavy degeneration in PTA at least until postoperative day 3 as revealed by either Fink-Heimer procedure I or II. Conversely, lesions resulting from the same current parameters applied for 3 seconds produce slight, if any, deficits and little or no degeneration of fibers to the PTA after the same survival period. The results suggest that a neural feedback loop traveling through or near the extreme lateral hypothalamus plays a role in modulating the effectiveness of ascending taste pathways. The latter pathways may be significant in the regulation of appetitive behavior.

437 RECOVERY FROM HYPOTHALAMIC APHAGIA AFTER A SINGLE INTRAHYPOTHALAMIC INJECTION OF APOMORPHINE (APO). Eileen O'Loughlin*and Samuel M. Feldman. Department of Psychology, New York University, New York, N.Y. 10003.

To investigate the role of dopaminergic (DA) transmission in the regulation of food and water intake, APO was administered intrahypothalamically to rats exhibiting the lateral hypothalamic (LH) syndrome. Aphagia and adipsia were induced by passing 0.5 ma DC between a LH anode and a rectal cathode for 30 sec on each side. Bilateral 23 g cannulae were then implanted with tips at the same stereotaxic coordinates as those of the lesion electrodes. Food and water intake and body weight were measured daily beginning one week prior to surgery. Only rats that were aphagic and adipsic, even when offered wet and palatable foods, were studied. Bilateral APO injections (10 µg/µl in distilled water, 1 µl/side) were administered at varying intervals (3 to 8 days) following surgery. Control injections included distilled water and norepinephrine. The major finding was that aphagia following LH damage was reliably and permanently reversed, within 20 to 26 hrs, by a single bilateral hypothalamic injection of APO. Following injection rats often used unusual tactics to approach food and drank from open dishes even though they could not negotiate water spouts. Recovery from adipsia was less dramatic because drinking from a water spout lagged behind acceptance of dry food pellets by 1-5 days, and rats remained somewhat hypodipsic. The results are not readily explained as the result of denervation hypersensitivity or acute transmitter replacement (Ljungberg & Ungerstedt, 1976), but rather suggest some diaschitic process similar to that reported by Marotta et al. (1975), who found that single IP injections of DA agonists reversed the septal syndrome. Ongoing research suggests that IP injections of DA or APO have similar effects to intrahypothalamic APO. The effect of varying dose and route of administration of several DA agonists is under investigation.

438 TASTE RECEPTORS AND GAIN OSCILLATION: A MODEL SYSTEM. Elizabeth Omand* and Jacob Zabara. Department of Physiology and Biophysics, Temple University, Philadelphia, Penna.

Studies were undertaken to examine position variability of taste receptors in order to extend an evaluation of the set point role in relation to the continuing systems analysis of fly feeding behavior included in this report.

Single unit recordings were made from from Phormia receptors by placing a micropipette containing a stimulating solution and conducting electrolyte over the tip of a peri-oral taste hair containing the minutely exposed terminals of a few chemosensory cells. Only the 'largest' hairs were used.

The variation observed within this receptor population was greater in older, more responsive, (unfed) animals. Middle hairs tended to have more responsive salt and sugar receptors; posterior hairs, lesser. Water receptors were least responsive in anterior hairs, most responsive in posterior hairs, with an ascending series in between. Each animal differed from this pattern in one or more receptors.

These data are interpreted in a systems analysis model as reflecting gain phenomena in the feeding neural system. The receptor sensitivity is a valid indicator of gain which is an almost linear function of time exhibiting a discontinuous boundary. Feeding resets individual receptor sensitivity and gain to a base level which remains constant until behavioral tracking time threshold is exceeded. Thus receptor variability, a readily observed experimental phenomenon, is an important component of nervous system state and output functions in this model.

439 CENTRAL INTERACTION OF PROSTAGLANDINS AND ANGIOTENSIN II ON DRINKING, BLOOD PRESSURE AND ADH RELEASE. M. Ian Phillips, J. Phipps*, W.E. Hoffman*. Neurobehavior Lab. Dept. Physiology, Univ. of Iowa, Iowa City, IA 52242.

Angiotensin II (AII) injected directly into the ventricles produces a drinking response, blood pressure increase and ADH release. There is evidence that AII releases prostaglandins in the periphery and that PGE_1 inhibits drinking to AII. The aim of this experiment was to test the effects of AII when prostaglandin synthesis was inhibited. Drinking in 6 rats after pretreatment with 1250 ng meclofenamate injected intraventricularly (IVT), AII (200 ng in 2 µl, IVT) produced a significantly greater amount of water intake over 15 minutes ($p<.002$). Individual data showed both the amplitude and the length of the dipsogenic response were increased. As a control for AII, carbachol was injected (200 ng IVT) after the same pretreatment. Drinking to carbachol (n=14) was almost abolished. As a further control, meclofenamate was injected i.v. (10 mg/kg) but had no effect on either AII (n=6) or carbachol (n=4) drinking. Blood pressure and ADH release: meclofenamate pretreatment had no effect on AII (50 and 500 ng IVT) induced pressor responses (n=15) but significantly increased the amount of ADH released ($p<.02$). The same pretreatment before carbachol (25 ng IVT) significantly lowered pressor responses ($p<.01$) ADH release ($p<.002$) and heart rate ($p<.01$). It is concluded: 1) that prostaglandin synthesis occurs in the brain when AII is injected, 2) that prostaglandins have a role in inhibiting AII drinking and ADH release responses but not pressor effects, and 3) central carbachol effects may be mediated by prostaglandins in the brain. (supported by NSF grant #BNS75-16346 and NIMH RSDA grant #3K02-MH70983-0151 to MIP)

440 THE PRESENCE OF A 4-DAY CYCLE IN FEEDING BEHAVIOR PRIOR TO PUBERTY IN THE FEMALE RAT. G.C. Sieck*, D.M. Nance, J.A. Ramaley, A.N. Taylor and R.A. Gorski. Dept. Physiol. & Biophys., Univ. Nebr., Omaha, NE 68105 and Dept. Anat. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

Feeding patterns of adult female rats are modulated by endogenous cyclic alterations in ovarian steroid levels during the 4-day estrous cycle. Since these rhythmic changes are thought to begin at puberty, i.e., vaginal opening (VO), we examined the development of feeding patterns in female rats from weaning (21 days) through the first completed vaginal estrous cycle. Animals were housed under a 12 hr:12 hr light/dark schedule in metabolic cages equipped with feeding tunnels and photo cells across the front of the food cups. A high fat chow and water were available ad lib. Food intake (FI) and body weight (BWt) were measured daily. Photo beam interruptions were continuously recorded and scored each day for total eating time (TET), number of meals (NM), meal size (MS) and meal duration (MD). Consecutive daily values of FI/100g BWt, BWt gain, TET, NM, MS and MD for individual animals during this period were analyzed for inherent rhythmicity using a fast Fourier transform spectral analysis. In each animal, a 4-day periodicity was found for all parameters of feeding behavior. Because the changes contributing to this periodicity appeared to correlate with VO, data from individual animals were aligned to this reference and statistically analyzed. Significant changes in each parameter commenced 8 or 12 days before VO and recurred every 4 days. Ovariectomy abolished the 4-day periodicity in FI/100g BWt and BWt gain suggesting that the ovaries are necessary for prepubertal cyclicity in at least these two parameters. These data reveal the presence of a 4-day periodicity in feeding behavior prior to puberty and support the potential role of ovarian hormones in these cyclic patterns. (Supported by NIH grants AM 18254, HD 08703 and NS 09122.)

441 SEXUAL BEHAVIOR OF OBESE RATS WITH ELECTROLYTIC OR GOLD THIOGLUCOSE IMPLANT HYPOTHALAMIC LESIONS. C. J. V. Smith*, D. L. Britt*, Y. M. Johnson* and L. L. Baranowski-Kish* (SPON: A. V. McGrady). Dept. Biol., Univ. of Toledo, Toledo, Ohio 43606.

Obesity induced by hypothalamic lesioning has been reported to produce disturbances in sexual behavior of mice and rats. Genital atrophy, persistent estrus and complete inhibition of mating behavior are among the changes that have been observed.

In this study sexual behavior of both male and female obese rats was examined. Animals were made obese by either gold thioglucose hypothalamic implants or electrolytic lesions of the ventromedial nuclei. Testing was done using normal, sexually proven animals.

The results indicated that the obese animals compared favorably with the non-obese control animals. Normal estrus cycles, lordosis, intromission, conception and parturition of young were all observed in the obese animals. Only slight differences were noted between the gold thioglucose and electrolytic lesioned animals.

442 THE EFFECTS OF CHOLECYSTOKININ ON FEEDING AND DRINKING IN GENETICALLY OBESE MICE. A.J. Strohmayer*, Glenn Kreielsheimer* and G.P. Smith. Dept.of Psychiatry, Cornell U. Med. Coll. and E.W. Bourne Behavioral Research Laboratory, N.Y. Hosp-Cornell Medical Center, White Plains, NY 10605

On the basis of parabiosis experiments, Coleman (1973) suggested that obese hyperglycemic mice do not release a humoral satiety factor, but are sensitive to it. We have considerable data that is consistent with the hypothesis that cholecystokinin (CCK) is a satiety factor in rats. To determine if CCK might be the circulating satiety factor, that obese mice do not release, the effect of CCK (5,10,20 and 40 units/kg, i.p.) on intake of liquid food (GIBCO EC 116) was measured in 6 lean (C57Bl/6J) and 6 obese (ob/ob) mice after 1.5 or 4.5 hours of food deprivation. CCK suppressed feeding in both obese and lean mice. The suppression was large: after 1.5 hours deprivation 40 units/kg suppressed feeding in obese mice 75% ($p<.025$) and lean mice 57% ($p<.025$). After 4.5 hours deprivation, 40 U/kg suppressed obese mice 84% ($p<.01$) and lean mice 94% ($p<.01$). The threshold dose for suppression of feeding was different for obese and lear mice and varied as deprivation time increased. After a 1.5 hour deprivation, the threshold dose was 10 units/kg ($p<.05$) for obese and 40 units/kg ($p<.025$) for lean mice. After a 4.5 hr deprivation, threshold was 20 units/kg ($p<.05$) for obese and 10 units/kg ($p<.025$) for lean mice. To determine if the inhibitory effect of CCK was specific for feeding, the effect of drinking was investigated in the same mice. After 4.5 hr deprivation, CCK (40 U/kg), had no effect on drinking in lean mice but significantly reduced the volume of water ingested (75%) in obese mice. These data demonstrate that the inhibitory effect of CCK was specific for feeding in lean mice but not in obese mice. These results suggest, but do not prove, that CCK is a satiety factor in C57Bl/6J mice and that the obese mutant is as sensitive to the satiety effect of CCK as lean mice. (Supported by the Lineberry Fund and NIMH Fellowship MH05385).

443 PERIOVULATORY PHENOMENA IN PREGNANT AND NON-PREGNANT RATS
Freya Weizenbaum* and Nancy J. Kenney. Dept. Psych, Va Polytech. Inst. & St. U., Blacksburg, VA 24061, & Inst. Neuro. Sci., U. of Pa Phila, PA. 19174.

Endocrine changes associated with pregnancy and parturition have significant effects on the ingestive behavior of the female rat. Rats gradually increase water intake during pregnancy until just prior to birth, when water intake drops precipitously. The timing and the pattern of this decrease is related to the total length of the gestation period. Rats giving birth 22 days after mating show a significant decrease in water intake on the day of birth. Rats that delivered 23 days after mating show the decrease in water intake on the day before birth as well as in the day of birth. Since the female rat ovulates shortly after birth (ENDO 76: 620, 1965); water intake patterns in the peripartum rat were compared to intake patterns in unmated female rats. Cycling females show marked decreases in water intake which are associated with ovulation. During a 4-day estrous cycle water is reduced in proestrus and during a 5 day cycle water intake is low on diestrus II and proestrus. Changes in food intake corresponding to those in water intake are seen in both the peripartum and cyclying rat. Thus the data show that the periovulatory intake patterns of rats who give birth on day 22 or 23 mimic those of the 4 and 5 day cycle rats respectively. Others have shown that the neuroendocrine events which control ovulation in the cycling rat also control ingestive behavior (PHY. BIOCH. BEH. 3: 155, 1975). Thus, the present experiments suggest that the neuroendocrine events which control ovulation and water intake in the cycling rat may be similar to those which control ovulation, water intake and length of gestation in the pregnant rat. Supported by GMS 218 to the Inst. Neuro. Sci. (F.W. & N.J.K.) and HDO 2273 (N.J.K.)

444 THIAMINE DEPRIVATION INDUCED ANOREXIA AND CONDITIONED TASTE AVERSION: THE CONTRIBUTION OF DIETARY FAT AND CARBOHYDRATE IN THE VMH RAT.
Robert M. Zacharko*, and Thomas B. Wishart. Dept. Psych., University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OWO

Animals with ventromedial hypothalamic lesions (VMH) develop anorexia sooner than controls when placed on a thiamine-free diet (Agnew & Mayer, 1956). High-fat, thiamine free diets significantly delay the onset of anorexia in normal rats (fat-sparing action). In this experiment, the sparing action of a high fat diet was determined in VMH animals. Normal controls and VMH rats had access to either a high fat (50%)-low carbohydrate (22%) or a low fat (6%)-high carbohydrate (66%) diet ad libitum. Both diets were thiamine free and subjects were supplemented with thiamine injections intraperitoneally (.25mg/rat/day) during adaptation, the hyperphagia test (12 days), and the recovery period. Thiamine injections were withdrawn during the deficiency period (48 days). Latency to anorexia was calculated for each animal. Thiamine deficient VMH rats fed for a significantly longer time on the high carbohydrate diet (29 days) than did thiamine deficient control rats (24 days). Conversely, thiamine deficient VMH rats developed anorexia significantly sooner than their respective controls on the high fat diet (24 days as compared to 28 days). All subjects except the VMH rats fed a high fat diet resumed normal feeding following thiamine supplementation (conditioned taste aversion). It was concluded that low fat diets have appetite sustaining functions for thiamine deficient VMH rats while high fat diets are deleterious. In the former case hyperphagia is an asset while in the latter case hyperphagia places these animals at a disadvantage since the VMH rat is not optimally suited to the utilization of high fat diets which it prefers. VMH lesions appear to render the rat more susceptible than controls (under high-fat, thiamine-free diet conditions) to the establishment of a conditioned taste aversion.

Invertebrate Neurobiology

445 MODULATION OF A SIMPLE REFLEX IN APLYSIA CALIFORNICA BY AROUSAL WITH FOOD STIMULI, C.Advokat*, T.Carew and E. Kandel, Dept. of Physiology, P & S, N.Y., 10032

The influence of arousal on reflex responsiveness has been examined principally in complex behavioral systems (Dethier, 1966; Kupfermann, 1974). We have examined the role of arousal in the simple defensive withdrawal reflex of Aplysia. In previous studies arousal produced by noxious stimuli has been shown to enhance reflex siphon withdrawal (Pinsker et al., 1973). We now find that arousal due to appetitive stimuli depresses this reflex.

We first examined the effect of food ingestion on the siphon withdrawal reflex. Two groups of animals were fed daily. Siphon withdrawal was examined at different intervals after the meal. One group (N=14) tested immediately after the meal exhibited significantly depressed reflex responsiveness ($p < .001$) compared to the other group (N=13) tested 24 hours after feeding. One week later, the conditions of the two groups were reversed and the results were the same (see Fig. 1).

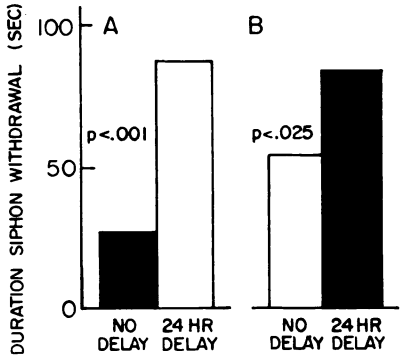


Figure 1. Reflex Depression After Feeding. Part A: Mean duration siphon withdrawal (sum trials 1-10, iti = 30 sec) for two groups of animals: one (solid bar) tested immediately (no delay), and another (open bar) tested 24 hr (24 hr delay) after a meal. Part B: One week later the conditions were reversed for the same two groups. Significance levels are indicated.

We next examined whether reflex depression requires ingestion. Three groups of animals were fed daily. One group (N=10) was tested immediately after feeding. A second group (N=9) was not fed and was tested after 15 min of food stimulation to the oral veil. A third group (N=9) was tested 24 hours after feeding. Both ingestion and food stimulation produced significant response depression ($p < .01$ and $p < .05$ respectively). Additional control experiments suggested that the chemosensory properties of food were critical for the depression since biting elicited by non-nutritive stimuli did not produce this effect.

These results and those of Susswein et al., 1976, suggest that arousal is not a unitary process in Aplysia. Arousal produced by noxious of appetitive stimuli can have opposite effects on different response systems and each of the arousal stimuli can have opposite effects on the same system. Thus, noxious stimuli that enhance siphon withdrawal depress biting (Kupfermann & Pinsker, 1968) whereas food stimuli that depress siphon withdrawal enhance biting (Susswein & Kupfermann, 1975). These findings support the suggestion from cellular experiments (Weiss et al., 1976; Hawkins et al., 1976) that even though the mechanism of the sensitizing component of arousal may be similar, the anatomical loci are separate and independent.

446 FINE STRUCTURE OF SYNAPSES OF IDENTIFIED SENSORY CELLS WHICH MEDIATE THE GILL-WITHDRAWAL REFLEX IN APLYSIA CALIFORNICA, C.H.Bailey*, E.B. Thompson, V.F.Castellucci, E.R.Kandel, Div. Neurobiol. & Behav., Col. P & S, Columbia Univ., N.Y., 10032

A major limitation to the morphological study of connections between identified cells in invertebrates has been the complexity of the neuropil. Recent development of suitable markers makes possible the ultrastructural study of synapses belonging to identified neurons. These techniques can now also be used to study morphological changes associated with behavioral habituation and sensitization. A recent electrophysiological analysis of these two forms of behavioral plasticity in the gill-withdrawal reflex of Aplysia has demonstrated that these modifications alter the quantal output of presynaptic terminals of the mechanoreceptor sensory neurons. It may now be possible to compare the morphology of these sensory synapses in the untrained, short-term and long-term habituated states. As a first step toward this objective we have labeled the cell body, neuropil processes and synapses of mechanoreceptor sensory neurons by the intrasomatic injection of horseradish peroxidase (HRP) (Muller and McMahan, Anat. Rec., 1975). Sensory somata are small (35-50 μm) and display conventional intracellular organization. Glial invagination of the cell body is moderate and contributes to a slight constriction of the main axon as it leaves the soma before expanding to ca. 8-10 μm upon entering the neuropil.

Areas of presumptive synaptic contact typically occur between a single labeled sensory element and one contiguous unidentified process, and are characterized by straightened plasmalemmas bounding a slightly widened cleft (mean width 8-10 nm greater than the unspecialized intercellular gap). The synaptic cleft frequently contains a dense intermediate line. The polarity of electron-dense material attached to the cytoplasmic leaflets at these sites is difficult to establish in the presence of HRP. Asymmetry has been determined in these regions by the presence of vesicles near or touching the plasmalemma of either one or the other process.

Using this morphological criterion for the direction of transmission, preliminary observations suggest that the labeled sensory process is most commonly the presynaptic element. In such cases, the sensory processes are densely packed with a population of vesicles having a relatively uniform appearance. The sensory vesicles appear in thin sections as slightly elongate, smoothly contoured profiles (axial ratio 1.2, mean length 80 $\pm$ 1.3 nm S.E., mean width 64 $\pm$ 1.3 nm S.E.). In favorable preparations, where the HRP reaction product does not obscure their morphology, some of these vesicles appear electron-lucent.

Occasional instances are found in which the sensory element appears by above criteria to be postsynaptic. In these cases presumptive synapses occur on profiles of sensory neurons less densely packed with vesicles, and in some instances the adjacent process contains numerous synaptic vesicles in addition to the ones which are close to the synaptic specialization. Although reconstruction of synaptic regions will ultimately be necessary to determine the precise geometry of synaptic contacts involving sensory processes some of the configurations in which sensory processes are apparently postsynaptic may represent the locus of synaptic input which causes heterosynaptic facilitation.

447 EVIDENCE FOR AN ENTRAINABLE CIRCADIAN OSCILLATOR IN THE ABDOMINAL GANGLIA OF CRAYFISH. Gene D. Block. Department of Biological Sciences, Stanford University, Stanford, CA. 94305.

Several circadian oscillations have been reported in crayfish. Both ERG and sustaining fiber activity display a circadian rhythm in response to light pulses delivered to the retina (Arechiga & Wiersma, 1969). In addition, locomotor activity appears to be under the control of an endogenous circadian oscillator (Page & Larimer, 1972). Data obtained from isolated abdominal ganglia demonstrate that the abdominal ganglia contain an entrainable circadian oscillator. If the abdominal ganglia and associated ventral cord are isolated and placed into darkness in culture medium a circadian rhythm is present in the frequency of spontaneously occurring multiunit activity recorded from the second root of the sixth abdominal ganglion. This circadian rhythm freeruns for at least three cycles and peak spike activity occurs near projected dawn of the previously applied lightcycles.

Adult crayfish (Procambarus clarkii) were exposed to lightcycles consisting of 12 hours of light followed by 12 hours of darkness. Half of the crayfish were placed on lightcycles with time of dawn at 14:00 PST while the remaining half experienced dawn at 22:00 PST. After exposure to at least four complete lightcycles abdominal ganglia 2 through 6 were dissected out as a unit and placed into culture medium (modified Eagles). Electrophysiological recordings were made by means of suction electrodes attached to the second roots of sixth abdominal ganglia. Preparations were maintained in darkness at constant temperature (16.5^{o}C). Four preparations, two from each lightcycle, were run simultaneously. Multiunit activity from the second root was electronically counted and printed onto paper tape each hour. A particular preparation was considered in the data tabulation only if it remained electrically active for at least 72 hours. Approximately 50% of the preparations met this criterion. The hour containing the maximum number of spikes during the second circadian cycle was used as a phase reference point. When in vitro activity peaks were plotted in relation to the time of dawn of lightcycles experienced in vivo it appeared that the abdominal oscillator was entrained by those lightcycles. Mean peak activity for animals exposed to 14:00 PST dawns occurred at 15:00 PST (n=11), whereas mean time of peak for crayfish exposed to 22:00 dawns occurred at 24:00 (n=7).

The ganglionic location of the circadian oscillator is presently unknown. Preliminary recordings from second roots of other abdominal ganglia also reveal circadian modulations. The possibility of more than a single circadian oscillator in the abdominal ganglia opens up the possibility of studying the coupling between circadian elements.
Supported by Postdoctoral training grant 1F32NS5035-01 & MH22114-05.

448 IONIC MECHANISMS CONTRIBUTING TO INKING BEHAVIOR IN APLYSIA. J. Byrne, N. Dieringer*, J. Koester and E. Shapiro*. Dept. of Physiol., Columbia Univ. and Dept. of Physiol. Univ. of Pittsburgh.

The release of ink from the purple gland in response to noxious stimuli is mediated by 3 electrically coupled motor neurons, $L14_{A,B,C}$ in the abdominal ganglion of Aplysia californica (Carew and Kandel, 1976; in preparation). Carew and Kandel found that electrical stimulation (6Hz, for 5 sec) of the pleuroabdominal connectives which mimics a noxious stimulus to the head typically produces an EPSP pattern in the L14 cells consisting of an initial large depolarization, followed by a smaller, nearly steady level of depolarization, with a subsequent slow increase in EPSP amplitude. When the stimulus is supra-threshold for spikes in the L14 cells, the initial PSPs are relatively ineffective in firing the cell compared to the PSPs of similar synaptic current which occur later in the train. Thus a several second pause often occurs before the cells attain their maximum firing frequency and cause the release of ink. Using current-and voltage-clamp techniques we have analyzed the mechanism underlying this initial pause.

A 5 sec depolarizing constant current pulse applied intracellularly to one of the L14 cells was used to roughly approximate a 5 sec supra-threshold train of EPSPs. An initial rapid rise in membrane potential was followed by a slower depolarization which increased over a 1-2 sec period to a level where spikes were elicited (Fig. 1A). Immediately after a smaller depolarizing prepulse, the slow rise and pause observed with the test pulse were eliminated, and spikes were elicited immediately (Fig. 1B).

Voltage clamp experiments with the L14 cells revealed that they have at least 3 ionic conductance mechanisms similar to those described by others in gastropod nerve cells. When the membrane is depolarized with gradually increasing voltage steps from resting level (-70mV) currents are activated in the following sequence: (1) a fast transient outward current, with a threshold at about -50mV; (2) a fast transient TTX-sensitive inward current, with a threshold at about -40 mV; (3) a slower, TEA-sensitive outward current (delayed rectification) which turns on at about -30 mV.

As pointed out by Carew and Kandel, the L14 cells are unique in that their resting potentials are about 20-30 mV more hyperpolarized than the typical value for Aplysia neurons. This insures that the level of inactivation of the conductance channels for the fast outward current will normally be low. Thus a train of EPSPs or a depolarizing current pulse can activate this current maximally thereby counteracting the initial effectiveness of the excitatory input (see Daut, Nature New Biol., 246, 193, 1973). This interpretation is supported by evidence from voltage clamp experiments. A test depolarization from resting level to about spike threshold resulted in activation of the fast outward current; it then decayed with a rate similar to the rate of depolarization in the current clamp experiment (Fig. 1C). This current was eliminated when the conductance channels were inactivated with a 5 sec pre-pulse (Fig. 1D).

These results suggest that a unique combination of biophysical properties of the L14 cells cause them to act as low-pass filter in the reflex pathway for inking. Their high resting potential, which insures minimal inactivation of the fast transient outward current channel, makes these cells preferentially responsive to long lasting stimuli.

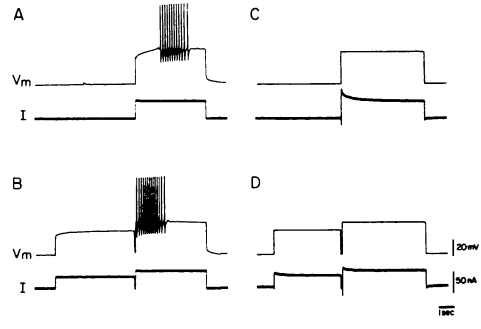


Fig. 1. A,B - Current clamp; C,D - Voltage clamp.

449 QUANTITATIVE ANALYSIS OF THE CONTRIBUTION OF CENTRAL AND PERIPHERAL NERVOUS SYSTEMS TO THE GILL WITHDRAWAL REFLEX IN APLYSIA CALIFORNICA T. Carew, V. Castellucci, J. Byrne and E. Kandel. Dept. of Physiology, College of Physicians & Surgeons, 630 W. 168th St., N.Y., 10032

There is now general agreement that the gill withdrawal reflex to weak tactile stimuli is entirely mediated by the CNS (abdominal ganglion) whereas the reflex to strong or noxious stimuli also involves peripheral pathways. Thus Peretz et al (1976), utilizing a 30 ms mechanical tap to the siphon skin, found that the reflex was centrally mediated for weak stimuli (1 g/ cm^2, applied to 1.8 mm^2 of skin) whereas strong stimuli (up to 1,130 g/cm^2) involved peripheral pathways. This conclusion is similar to that reached by Kupfermann et al (1971). There is however disagreement on the consequences of moderate intensity stimuli. Kupfermann et al and Kandel et al (1976) found that the abdominal ganglion mediates 94% of the withdrawal reflex to moderate intensity 800 ms water jet stimuli (250 g/cm^2, applied to approximately 300 mm^2 of siphon) and to a behaviorally comparable 800 ms probe stimulus (1,800 g/cm^2, applied to .25mm^2). But Peretz et al found in this stimulus range that the reflex amplitude was on average unaltered when the CNS was removed.

In two joint experiments with Peretz, Jacklet and Lukowiak, using the upper range of moderate intensity stimuli, we inferred that differences might result from the type of preparation and stimuli used, and the criteria for accepting responses. Kupfermann et al used intact animals which exhibited near maximal responses to moderate intensity jets of seawater, and accepted preparations which showed responses that were at least 35% of maximal spontaneous pumping movements. Peretz et al used an isolated gill-mantle preparation and did not exclude preparations that showed small reflex responses.

To see if these differences explain the discrepancy, we videotaped the reflex elicited by water jets of moderate intensity (250g/cm^2) in relatively intact animals. Whereas sham operation (N=10) reduced the reflex to 98.3% of control, deganglionation (N=10) reduced it to 10.1%. Using a protocol developed jointly with Peretz et al., we next examined the reflex with a strain gauge in isolated preparations, and alternated a controlled force probe to the rim of the siphon (Byrne et al., 1974), and a "tapper" stimulus to the base (Peretz et al., 1976). A single force of 4.0 g (the upper range of moderate intensity) was used (probe diam. 0.56 mm: p = 1600g/cm^2; tapper diam. 1.5 mm: p = 228g/cm^2). Preparations were accepted only if probe responses were, at the outset, at least 35% of maximal spontaneous contractions (54% of preparations), but, as in Peretz et al., 1976, no response amplitude criteria were imposed on the tapper. Control responses to the probe were 72% and to the tapper 9% of maximal. Deganglionation (N=10) reduced the responses to probe and tapper to 5% and 2% of control. Experiments (N=10) in which the ganglion was not excised indicated that neither habituation nor deterioration account for these results.

In additional experiments (N=7) we imposed no criterion on the probe responses: the mean control response was 13% (probe) and 2% (tapper) of maximal. Now the results were clearly different. Whereas the mean probe response after deganglionation was again small (14% of control), the mean tapper response was 121% of control.

In summary, the CNS mediates at least 90% of the reflex to moderate intensity water jet stimuli in the intact animal and at least 95% in the surgically isolated preparation to probe and tapper stimulation when minimal response criteria are used. Although the CNS still mediates at least 86% when small reflex responses to the probe are examined the small responses to the tapper are on average unaltered when the CNS is removed. Thus one can examine the reflex as mediated predominantly by the CNS or by the peripheral system by appropriate choice of stimulus intensity, duration, location, and particularly, magnitude of reflex responsiveness.

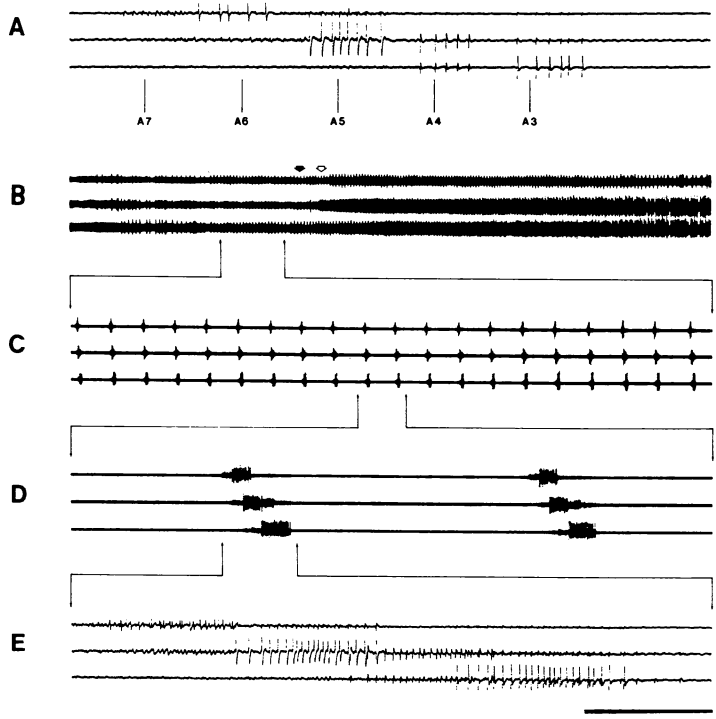
450 THE NEUROPHYSIOLOGY OF ECDYSIS IN ORTHOPTERA (INSECTA). J. R. Carlson* (SPON: G. Hoyle). Dept. of Biology, Univ. of Oregon, Eugene, OR 97403

The ecdysial behavior of a cricket (Teleogryllus oceanicus), and the locusts (Schistocerca gregaria and S. vaga) has been studied by cinematography and videotape analysis. Ecdysis consists of 3 functionally distinct phases common to each species: (1) preparatory - loosening the old cuticle (2 hrs), (2) ecdysial - escape from old cuticle (20 min), (3) expansional - inflation of new cuticle (60 min).

The behavior occurs as a regular series of bouts of activity which alternate with periods of quiescence. As ecdysis progresses, independent behavioral components are recruited in stereotyped sequences characteristic of each species. In the cricket, 48 of these components were found: 6 preparatory, 30 ecdysial, 8 expansional, and 4 during the additional period when the cricket eats its old exoskeleton.

Due to the simplicity of the insect neuromuscular system, in which each muscle is supplied by a small number of motoneurons (often one or two excitatory neurons), it was possible to describe certain of these behavioral components quantitatively in terms of the activities of a few identified motoneurons which were continuously monitored by electromyography (Fig. illustrates the motor pattern underlying peristaltic waves of abdominal shortening in the cricket where a single motoneuron controls each muscle. Time mark = 0.3 s).

In addition to its value as a model system for studies of complex, long-lasting, stereotyped behaviors, Orthopteran ecdysis may be hormonally triggered, as is the homologous eclosion behavior of the saturniid moths. This would permit the study at the level of identified neurons of the mechanism of hormone action in triggering a behavioral sequence. (Supported by USPHS grants NS09074, 5-T01-GNO-1021-11, and 1-F32-MH05126-01).



451 MOLLUSCAN SALIVARY NEURONS: CHARACTERIZATION AND MODULATION DURING FEEDING. Jonathan Copeland*, Barbara Symonds*, and Alan Gelperin (SPON: R. Olivo). Dept. Biology, Princeton University, Princeton, New Jersey 08540.

We have used a combined neurophysiological, histological, and behavioral approach to study the neural control of salivation, one aspect of the feeding behavior of the garden slug Limax maximus.

Limax possesses paired buccal ganglia, salivary glands (SGs), and contractile salivary ducts (SDs) which deliver saliva to the oral cavity in the buccal mass. Salivary flow is greatly enhanced during feeding. Each buccal ganglion innervates the ipsilateral SD and SG by a salivary nerve.

Staining of salivary gland neurons (SNs) by axonal iontophoresis of cobalt reveals 8 - 17 somata in each buccal ganglion. The three largest SNs (soma diameter 150 μ) lie near the entrance of the gastric nerve (GN). Cobalt and methylene blue staining demonstrate 25 - 50 cells (soma diameter 50 μ) occurring in clusters at branch points of the salivary nerve and duct on the surface of the SG. Transmission EM of the salivary nerve shows over 3000 axon profiles, 91% of which are 1 μ or less in diameter. Five large axons (diameter 6.5-10 μ), characterized by deep infoldings of the axonal membrane, are present in the salivary nerve. The epithelial cells facing the lumen of the SD have microvilli on their luminal surfaces and are coupled together by tight junctions.

Simultaneous extracellular recordings from both salivary nerves allow unequivocal identification of 3 spontaneously active SNs in each salivary nerve. One of these is an autoactive motoneuron with an ipsilateral soma, the salivary burster (SB), which initiates duct peristalsis[1]. The remaining two cells each have an axon in both salivary nerves. These bilateral salivary neurons (BSNs) can be discriminated because the axon ipsilateral to the soma produces a larger extracellularly recorded spike than the contralateral axon.

During feeding each SB is synaptically modulated via electrotonic synapses with protractor motoneurons so that its activity is phase-locked to the protraction-retraction cycle[1]. The BSNs also show increased activity during feeding but are not modulated cycle by cycle.

The SB, BSN, and 2 additional large salivary neurons (SN1 and SN2) in each buccal ganglion have been identified by intracellular recording. Each of these cells is synaptically modulated by the metacerebral giant cell (MGC), an identified serotonergic neuron which is activated during feeding. Each MGC has an axon in the salivary nerve, inhibits SB, and excites BSN, SN1, and SN2.

We are currently investigating the effects of MGC on the large buccal ganglion salivary neurons and the roles of these cells and the peripheral cluster cells in the secretion, transport, and resorption of saliva.

(Supported by NIH fellowship 1 F32 NS05067-01, the Spencer Foundation, and NSF grant BMS74-03572.)

[1]Gelperin, A. and D. Forsythe. In press. Neuroethological Studies of Learning in Mollusks, Chapter 16. In: J.C. Fentress (ed), Simpler Networks: An Approach to Patterned Behavior and its Foundations. Sinauer Associates, Sunderland, Mass.

452 CHEMOSENSORY INPUTS TO AN IDENTIFIED INTERNEURON IN A TERRESTRIAL MOLLUSC. Michael E. Egan*and Alan Gelperin. (SPON: Roger Cholewiak). Department of Biology, Princeton University, Princeton, NJ 08540.

Each metacerebral lobe of the cerebral ganglion (CG) of the giant garden slug, *Limax maximus*, contains a giant neuron (MGC) that is homologous to interneurons found in several molluscan species. In *Limax* this cell is active during feeding behavior (1-2.5 spikes per second) and has been shown to synapse on interneurons active during this behavior, presumably in a modulatory manner.

An epithelial pad, presumptive sensory neurons, and a digitate ganglion (DG) occur at the tip of each superior (optic) tentacle. The olfactory nerve (ON) travels between the DG and the CG. Staining of axons in the ON by cobalt iontophoresis towards the CG reveals three distinct fiber tracts; none of these tracts leave the CG, nor do they cross the cerebral commisure. One tract terminates in a circular arborization where the MGC characteristically lies.

The activity of the MGC and ON was examined in an isolated preparation (brain with superior tentacle attached) in response to odors. Odors were blown across the epithelial pad of the tentacle with a device which produces uniform pulses of odor that are contained in a constant stream of clean air; air flow rate, the duration of an odor pulse, and odor concentration are adjustable. Intracellular recordings revealed a low frequency train of spikes (1 Hz for a few seconds) in the MGC in response to a single 0.25 ml pulse of amyl acetate. This activity followed compound action potentials recorded en passant from the ON with a long latency (0.5 sec). Electrical stimulation of the ON produced compound synaptic potentials in the ispilateral MGC with the same long latency. Isolated tentacle-ON preparations responded to a variety of odors, including those from food items, with a short train of compound action potentials. Research is in progress to determine the relationship between MGC activity, sensory stimuli, and feeding behavior.

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453 CEREBRAL NEURONS MEDIATING A TENTACULAR REFLEX IN APLYSIA.
Steven M. Fredman and Behrus Jahan-Parwar. Worcester Foundation for Experimental Biology, Shrewsbury, MA. 01545

The neurons of the symmetrical B clusters in the cerebral ganglion of Aplysia exhibit high sensitivity to both mechanical and chemical stimulation of the ipsilateral and contralateral anterior (oral veil) tentacles. Merely disturbing the water near the tentacles is often sufficient to produce spiking in these neurons. Stronger mechanical stimulation gives a greater response, associated with it is a contraction of the tentacles. Tentacular movement is also usually exhibited by Aplysia during food (seaweed) sensing and following presentation of food extract to individual tentacles.

To investigate the relationship of B neuron activity and tentacular contraction, the CNS of Aplysia californica was placed in one compartment of a plexiglass chamber, and the anterior tentacles in separate, sealed compartments. Contraction of each tentacle was monitored with a force transducer connected by a fine thread to the tip of the tentacle. Mechanosensory stimuli were presented either by hand or by an electromechanically activated 1mm diameter glass rod. Sensory stimulations of either tentacle, sufficient to produce moderate to vigorous firing of the B neurons resulted in subsequent ipsilateral and contralateral tentacular contraction. Contractions were also obtained by passing depolarizing current through intracellular pipettes in 1 to 4 ipsilateral B neurons. Driving contralateral B neurons did not produce measurable contractions. Replacing the seawater in the CNS compartment with a solution containing 200 mM Mg^{++} and 0 mM Ca^{++} blocked all synaptic activity including that evoked by mechanosensory stimulation. This treatment failed to block contractions of the tentacle resulting from depolarization of impaled B neurons. Similarly, treating the tentacles with high Mg^{++} for 15-18 hours blocked all muscular contractions but failed to block excitation of B neurons produced by mechanosensory stimulation.

From these experiments it is concluded that pathways from mechanoreceptor neurons in the tentacles to B neurons act centrally and do not require peripheral synapses. B neurons act directly at the tentacles, not by polysynaptic pathways via other centrally located motor neurons. Although driven by bilateral sensory inputs, the effect of B neurons is seen primarily ipsilaterally. The B neurons can be considered to be presumptive motor neurons involved in reflex activity and control of tentacular movement.

This work was supported by PHS Grants NS 11452 and NS12483 to B.J.P.

454 MOLLUSCAN FEEDING MOTOR PROGRAM: RESPONSE TO LIP CHEMOSTIMULATION AND MODULATION BY IDENTIFIED SEROTONERGIC INTERNEURONS. Alan Gelperin and Joseph Jin Chang*. Department of Biology, Princeton University, Princeton, N.J. 08540

A preparation of the lips, cerebral ganglia and buccal ganglia is being used to study the effectiveness of lip chemostimulation in eliciting the feeding motor program in the terrestrial slug, Limax maximus. The right and left lips are sequestered in separate chambers and continuously superfused with saline or standard food extracts while we record from buccal and cerebral nerves extracellularly and selected neurons intracellularly.

The feeding motor program is characterized by a particular pattern of coordinated neural output from buccal and cerebral nerves which repeats at or close to the bite frequency of intact slugs (0.6 - 0.7 bites/sec.). Standardized extracts of very attractive food plants (carrot, cucumber, mushroom, potato, tomato) elicit vigorous rhythmic motor output. Thirty seconds of chemostimulation applied every twenty minutes yields reproducible responses in terms of response latency, frequency and duration for over three hours. Response latency is increased and frequency and duration decreased when stimulus strength is reduced by dilution of the standard plant extract. Less attractive feeding stimulants (lettuce extract, 2% soluble starch, 0.1 M monosodium l-glutamate) elicit very weak feeding responses or none at all.

Intracellular recordings from the serotonergic metacerebral giant cells (MGCs) during vigorous expression of the feeding motor program reveal few action potentials emitted by the MGCs during many seconds of feeding motor output. Discrete bursts of inhibitory postsynaptic potentials are observed in MGCs during the major protraction burst in buccal roots 1 and 3. These synaptic potentials restrict MGC activity to the periods before and after protraction. Stimulation of the MGCs alone rarely activates the feeding motor program, and when activation occurs, only 2-6 cycles of motor program are produced despite continued MGC activity at 1/sec. Driven MGC activity at 1/sec. concurrent with lip chemostimulation can increase the frequency of motor output during expression of the motor program.

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455 ON THE PRESYNAPTIC ORIGIN OF RIGHT-CONNECTIVE INPUTS TO APLYSIA NEURON R15. Peter Harley, Department of Psychology and Marine Sciences Research Laboratory, Memorial University of Newfoundland, St. John's, Newfoundland.

The right connective EPSP in neuron R15 is caused by transmitter release from an electrical syncytium of presynaptic terminals (all models, Fig. 1). With high-gain intracellular recording (200 μV/box), it is also possible to see a coupling potential or field effect associated with the incoming right connective volley (all models, Fig. 1). Anatomically, the simplest pathway which could account for all observations to date is model A. However, model A' must also be considered because there are small (10-20 μV) action potentials which appear spontaneously in the right connective, and such responses can be driven by stimulation of the siphon nerve. Moreover, the latency of the EPSP, as reflected in the Y-intercept of a latency-vs-distance plot, is long enough (say 10 msec at 14°) that two sequential synapses are not out of the question (models B and C). However, this estimate of delay necessarily contains the time required for development of the action potential in the immediate presynaptic apparatus, and if this region is assumed to cause loading of the right connective axons, then the latency estimate might well be considered supportive of a monosynaptic interpretation. In any case, Model B was not supported by a collision failure experiment which could have indicated the electrical integrity of initial right connective axons. Although no component of the right connective action potential associated one-for-one with the appearance of the EPSP, tests were performed to determine whether refractoriness and susceptibility to fatigue were homogeneous throughout the pathway. These experiments entailed pulse-pair stimulation of the connective at two sites, in either a far-near or a near-far sequence, while recording from R15. Because of the greater rest given the ganglionic side in a near-far sequence, it was possible to demonstrate that the absolute refractoriness of the whole pathway was equal to the absolute refractory period of the initially excited right connective elements (14 msec). Fatigue, however, builds up faster on the ganglionic side, developing in a fashion which resembles the behavior of a similarly treated axon with an electrical load on the near end of it (R2). This, in turn, is unlike the behavior of distal regions of the R2 axon and supports the idea that there is presynaptic electrical loading of the axons which excite R15. Supported by NRC grant A9792.

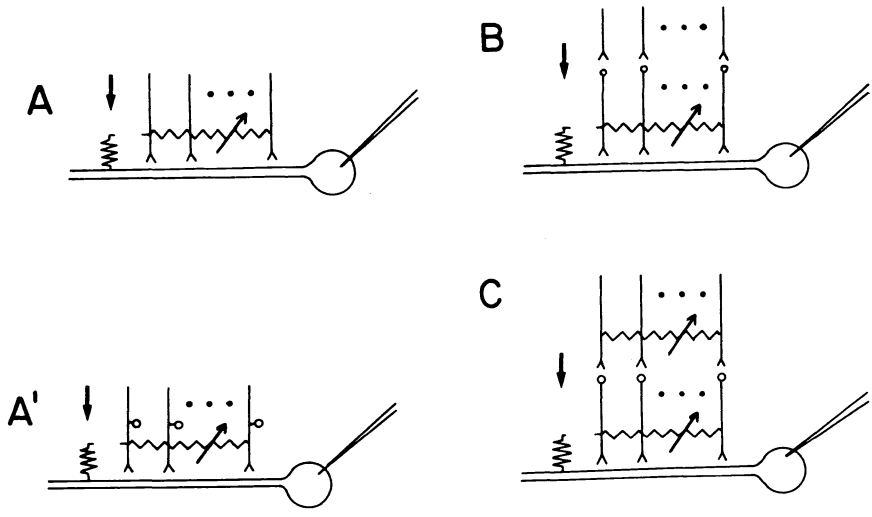

Fig. 1

456 QUANTITATIVE ANALYSIS OF PYLORIC NETWORK IN STOMATOGASTRIC GANGLION

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Final acceptance of qualitative explanations for patterned activity in simple nerve nets must depend on verification by quantitatively accurate models involving physiologically measured properties of the network. In addition, discrepancies between physiology and model predictions should reveal new phenomena which were not taken account of by the model. Given an accurate model, manipulations can be performed on it which cannot be performed on a physiological preparation (e.g. addition or deletion of synaptic connections), giving additional insight into system functioning. By parameter fitting the model within a known physiological range, it may be possible to deduce from extracellular records parameters or events normally only observable with intracellular techniques.

In the lobster (*Panulirus*) stomatogastric ganglion, a qualitative model for the main three-part activity sequence has been advanced: 1. an endogenous oscillatory driver potential (ca 1 Hz) depolarizes the 3 PD/AB cells, firing in which directly inhibits 1 LP and 8 PY cells. 2. when PD activity ends, the LP cell rebounds first, inhibiting the PD's and delaying the PY rebound. 3. when the PY's overcome the stronger inhibition from PD's and the weak inhibition from LP, they fire a burst, inhibiting the LP, thus disinhibiting the PD's and the cycle repeats. This model was subjected to quantitative test.

PD burst rate is increased (adapting over the next several seconds) by sustained depolarizing current. Short (50msec) depolarizing pulses caused slight increases in interburst interval (IBI) if place early in IBI, and substantial decreases if placed later. Hyperpolarizing pulses had the opposite effect, and in addition, readily terminated the burst (as did an LP IPSP) if place late in the burst, with a marked decrease in following IBI. A model involving an "on" and an "off" state with thresholds for transition between was used to simulate this.

Kinetic properties and synaptic interactions were measured for each of the three major cell types. Firing rate to a depolarizing step fell 50-70% of initial value along a compound exponential time course with time-constants 0.2-0.4sec and 3-6sec. Initial and final frequencies were linear with current over a moderate range (dropping at high and low ends). Post-hyperpolarization rebound was linear with current and followed a two-component exponential build up with the same time-constants as the adaptation. IPSP time-courses were measured for all synapses. Most (including AB onto LP, VD, and PY) were sharp rise (10-20msec to peak) and fall (τ = 40-80msec) but those from the PD (including onto VD) rose slowly ($\sim$80msec) and fell slowly (τ $\sim$80msec). Fast-rise PSP's were easy to reverse with hyperpolarization; slow-rise ones were not. Synaptic strengths were measured as the mean number of impulses which were eliminated from a post-synaptic train by a single presynaptic impulse. These were: PD-to-LP:-0.3; PD-to-PY: -0.5; LP-to-PD: -0.5; LP-to-PY: -0.2; PY-to-PD: -0.2 to -0.3.

These values were inserted in a 3-celled (PD,LP,PY) network model. Cell excitabilities (not directly measurable) were adjusted in model to fit physiological records. Significant discrepancies between model and physiology were: 1. failure of PY neuron to terminate activity immediately after start of PD burst; perhaps due to absence of sharp-rise IPSP's from AB (a giving possible function to the AB neuron); 2. adjusting LP excitability to fit observed frequency caused too much overlap with PY burst; perhaps due to absence of observed chemotonic inhibition of LP by PD; 3. PY burst started too soon after LP because PD + LP inhibition was too weak to delay it enough. This finding led to the discovery of a hyperpolarization-initiated hyperpolarizing conductance, normally triggered by PD-time inhibition, which delays the PY discharge. This requires a substantial revision of the original qualitative model. Quantitative model performance was substantially improved by addition of these effects.

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457 IDENTIFICATION OF INDIVIDUAL NEURONS MEDIATING THE HETEROSYNAPTIC FACILITATION UNDERLYING BEHAVIORAL SENSITIZATION IN APLYSIA, R. Hawkins*, V.Castellucci, E.R.Kandel, Dept. of Physiology, Columbia Univ., P & S, N.Y., 10032

A strong stimulus to the head of Aplysia can produce short-term or long-term sensitization of the gill-withdrawal reflex (Pinsker et al., 1970, 1973). Short-term sensitization is due to heterosynaptic facilitation which results in greater transmitter release at the excitatory synapses made by identified sensory cells on the gill motor cells. This facilitation can be produced in the isolated abdominal ganglion by stimulation of the connectives from the head ganglia. Connective stimulation also produces two other phenomena: (1) hyperpolarization of the sensory neurons and (2) increased concentration of cAMP in the ganglion. The facilitation can be simulated by perfusion of the ganglion with 10^{-6} M serotonin or by intracellular injection of cAMP in a sensory cell, and it can be reversibly blocked by perfusion with the serotonergic antagonist cinanserin (10^{-4} M) (Brunelli et al., 1976). These data suggest that sensitization is mediated by serotonergic cells that act on the sensory neuron terminals to hyperpolarize them and to increase intracellular levels of cAMP; cAMP in turn could enhance transmitter release perhaps by increasing the level of free Ca.

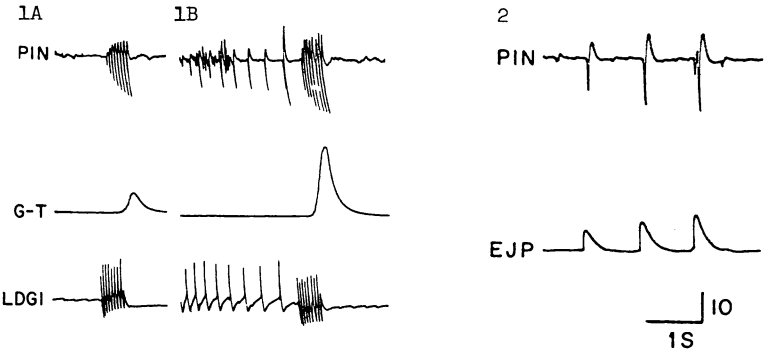
In order to further test these proposals we have tried to identify a cell or cells in the abdominal ganglion that mediate the sensitization. Consideration of the circuit diagram for the gill-withdrawal reflex suggested that facilitation might be mediated by one of the interneurons in the reflex pathway. We first examined the inhibitory interneuron L16 and found that it produces the hyperpolarization in sensory cells which accompanies facilitation by connective stimulation. However, direct intracellular stimulation of L16 does not produce significant facilitation. By contrast, we have found other cells which do not produce hyperpolarization but which do facilitate the EPSPs at the sensory-to-motor synapses. The facilitation can be produced by at least two independent cells in the same ganglion. In approximately half of the cases (N=18) the facilitating cells have had the properties of excitatory interneurons: they receive EPSPs from sensory cells and produce excitation in motor cells.

We have observed facilitation both of complex EPSPs (produced by siphon nerve stimulation) and of unitary, monosynaptic EPSPs (produced by intracellular stimulation of a sensory cell). The average amplitude of facilitation produced by a single cell is 150% above control (max = 500%) and the average half-life is 75 seconds. These values are less than for facilitation produced either behaviorally or by connective stimulation. One possible explanation of the discrepancy is that the combined activity of two or more cells may produce larger facilitation.

Preliminary work suggests that the facilitation produced by single cell stimulation is reversibly blocked by perfusion with 10^{-4} M cinanserin, which is consistent with the proposal that the facilitating neurons are serotonergic. Weiss et al. (1975 and this meeting) have provided evidence that serotonergic cells in the cerebral ganglia of Aplysia mediate a form of sensitization of the feeding response. This effect can be simulated by cAMP analogs. Comparison of these findings with our own suggests that the systems mediating sensitization of feeding and of gill-withdrawal are pharmacologically and biochemically similar, but that they are at least in part anatomically distinct.

458 FACILITATION OF EXCITATORY JUNCTIONAL POTENTIALS FROM GILL MUSCLE OF APLYSIA: CORRELATION WITH PINNULE POTENTIALS AND CONTRIBUTION TO HABITUATION. Jon W. Jacklet. Dept. Biol., SUNYA, Albany, N.Y., 12222.

Extracellular recordings (suction electrode) from the pinnules or veins of the gill (pinnule potentials, Peretz, 1970) have been used previously to monitor electrical events in the gill. Direct selective stimulation of a central motor neuron (LDG1) during simultaneous recording of gill pinnule potentials and gill tension shows that pinnule potentials (from efferent vein) are correlated one-one with action potentials recorded in the motor neuron (Fig. 1). The pinnule potentials systematically increase in amplitude (facilitate) with successive action potentials. Gill tension begins to increase only after considerable facilitation of the pinnule potentials has occurred. In Fig. 1B, antecedent activity of LDG1 (which does not produce tension) prior to the evoked burst of action potentials produces facilitation of the pinnule potentials. Then, the evoked burst of action potentials in LDG1 produces a much larger gill tension than before because the pinnule potentials start from a greater facilitated state. Intracellular recordings from longitudinal muscles of the efferent vein of the gill during simultaneous recording with the extracellular electrode show (Fig. 2) that the pinnule (efferent vein) potentials are caused by muscle excitatory junctional potentials (EJP). The amplitude of the pinnule potentials is an accurate reflection of the facilitation (homosynaptic) of the EJP. Gill muscles had resting potentials of 60-70 mv and a single fiber usually received more than one excitatory input and infrequently an inhibitory input in agreement with Carew et al., 1974.



As a consequence of homosynaptic facilitation at the gill neuromuscular junctions, the relative contribution of a central motor neuron action potential to gill tension and withdrawal will vary with previous activity in that neuron (Fig. 1). This facilatory influence contributed to short term adaptive responses such as habituation. A reduction in the action potential output of a central neuron during habituation (caused by synaptic depression at its input, Castellucci et al., 1970) would lead to a reduced effect of each action potential in gill withdrawal because of reduced facilitation of EJP's. On the other hand, an increase in output of action potentials between habituation trials, such as dishabituation, would serve to increase the effect of action potentials at the next habituation trial (i.e. Fig. 1B). The effect will depend upon the rate of firing of the motor neuron and the intertrial interval used in habituation. At intervals of one minute or less it contributes significantly considering the usual firing rate of central neurons during dishabituation. Supported by NIH 08443.

459 NEW MOTOR COORDINATING INTERNEURONS USED BY GIANT AND NON-GIANT ESCAPE SYSTEMS IN THE CRAYFISH, *PROCAMBARUS CLARKII*. Andrew P. Kramer* (SPON: Frank Krasne). Department of Psychology, UCLA, Los Angeles, Ca. 90024.

The well studied neural circuitry of crayfish reflex escape behavior is characterized by its simple design. Stereotyped backward-propelling tail flips are reliably produced by the firing of single, giant, "command" interneurons (the lateral (LG) and medial (MG) giants), which monosynaptically connect to appropriate phasic flexor motoneurons of the abdominal musculature.

In addition to this giant system, there is a class of complex, non-stereotyped tail flipping that does not utilize the giant interneurons and whose neural circuitry is poorly defined. I now report that the giant and non-giant systems are linked on the motor side by making common use of what I shall call phasic motor "coordinating interneurons." Besides being the first description of non-giant tail flip interneurons, this finding intimates that the motor control of the giants may be more complicated than now supposed.

Three bilaterally symmetrical pairs of giant/non-giant shared interneurons have been found, but only one (I3), which is the best studied, will be described; the others have somewhat similar characteristics. The I3's have dendrites and cell bodies in the third abdominal ganglion and a large (30-40μm) axon contralateral to the cell body and major dendrites, which runs caudally, just ventral to the MG's, to the sixth ganglion. Each I3 monosynaptically and bilaterally fires several telson phasic flexor motoneurons in the sixth ganglion, which momentarily flexes the abdominal tail fan. The I3 triggering stimulus is believed to be the firing of the third ganglion phasic flexor motoneurons. The evidence for this is twofold: (1) Aside from the giants and non-giants, the only effective I3 stimulus I have found is the stimulation of a peculiar first root axon which fires ipsilateral phasic flexor motoneurons of the third ganglion, and (2) antidromic stimulation of these flexor motoneurons can excite, though not fire, I3.

I3 is obligatorily fired by LG and MG but only sometimes fired during the flexion phase of non-giant tail flip responses. This suggests that the non-giant system may modulate the ability of the motoneurons to fire I3. Indeed, microelectrode recordings from I3 in a largely intact but restrained animal reveal that when the non-giant system is naturally stimulated below response threshold, I3 receives flurries of brief depolarizations. These become large, long (100ms) depolarizations, peaking during flexor firing, when a non-giant response occurs. These depolarizations are interpreted as a facilitatory priming produced by the non-giant system, which may be necessary for I3 to be triggered during a non-giant response.

The hypothesis is (1) that I3 functions to cause certain tail fan flexor motoneurons to fire at a specific moment in the tail flip motor program, namely, a few milliseconds after the third ganglion phasic flexor motoneurons have fired, and (2) that the non-giant tail flip control system permits or prevents I3 from performing this function depending on whether or not it is consistent with the planned response morphology. With this kind of control, a set of such motor interneurons, each coordinating a small piece of a tail flip sequence, would be ideally suited to play a role in structuring the complex non-giant response patterns. The firing of these motor interneurons during giant-mediated tail flips suggests that perhaps the direct giant to motoneuron connections are not as autonomous as has been supposed. Perhaps they function appropriately only when the motoneurons are co-activated by the coordinating interneurons described here.

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460 SUCCESSFUL REINNERVATION OF AN AKARYOTIC CRUSTACEAN NEURON BY REGENERATING AFFERENTS. F. B. Krasne & Sun Hee Lee*.(SPON: Lyle Rausch). Department of Psychology. UCLA. Los Angeles, Ca. 90024.

Whereas most neurons degenerate after removal of their somas, certain arthropod interneurons and motor neurons remain capable of firing in response to chemical synaptic input, propagating spikes, and releasing transmitter for months after removal of their cell bodies (and the nuclear genome therein). There thus arises the question of what functions are disrupted in such akaryotic neurons. A plausible possibility is that routine maintenance activities may persist without the soma, but novel activities outside the "normal line of duty" may not. Such thinking has led us to investigate whether regenerating sensory axons can establish functional contacts with a denervated sensory interneuron that lacks its cell body.

Interneuron A (hereafter A) of the crayfish abdominal nerve cord receives chemically transmitting terminals of tail fan tactile afferents that arrive over ipsilateral ganglionic roots 1-5. A's soma lies contralateral to its axon and dendritic field at the caudal margin of the ganglion and can be reliably disconnected from the neuron by a caudally placed midline incision. In 12 experimentals (A's soma disconnected) and 8 controls roots 2 and 4 were cut about 2 mm from the ganglion and the peripheral segments tied to root 3 (sometimes severed from the periphery and sometimes intact until the time of testing) which acted as a guide for growth of 2 and 4 toward the ganglion; ties were placed around the central stumps of 2 and 4 for later identification, and other ipsilateral roots were severed and allowed to retract.

The course of functional regeneration of input to A from roots 2 and 4 was essentially identical in experimentals and controls. There was no recovery seen in the first week after surgery, some in the second, and apparently full recovery by 5 weeks. Terminally, tactile stimulation of the sensory fields of roots 2 and 4 was shown to still be effective in firing A after (i) verifying the absence of regeneration of roots 1, 3, and 5 and (ii) cutting the tied central stumps of 2 and 4 so as to sever 2nd and 4th root fibers that might have re-united with their distal segments. Electrophysiological tests ruled out the possibility that apparent regeneration was due to fibers of roots 2 or 4 having fused peripherally with 3rd root fibers.

We conclude that akaryotic neurons can receive and reform functional synapses with terminals of regenerating afferents. The extent to which this involves active participation of the akaryotic target neuron remains to be determined.

461 RESPONSE OF GIANT AXONS OF THE COCKROACH (PERIPLANETA AMERICANA) TO WIND FROM DIFFERENT DIRECTIONS. J. J. Langberg*, J. Westin*, and J. M. Camhi. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, N.Y. 14853.

The cockroach Periplaneta americana has at least 8 giant axons in each of the two connectives of its nerve cord. Most or all of these are excited by sensory neurons innervating cercal hairs which are deflected by air displacement. Each hair has a preferred plane of deflection which confers upon its sensory cell a limited angular range of responsiveness (Nicklaus, R. Z. vergl. Physiol. 50: 331-362, 1965). Thus it is possible that the giant axons are also directionally selective.

To test this possibility, we developed a stimulator which delivered reproducible puffs of air to the cerci of a stationary cockroach. These puffs were laminar, with a 70 msec. duration and a 3 m./sec. peak velocity. (Higher peak velocities evoked no greater response in the cells tested.) The delivery tube could be rotated about the cerci in the horizontal plane. Changing the angle of delivery did not affect the internal conformation of the wind source.

Using a microelectrode filled with procion yellow, we recorded action potentials from a giant axon in response to wind from different directions. Subsequent iontophoresis of procion yellow allowed identification of the axon by fluorescence microscopy. Of 61 cells recorded, 31 were positively identified as cells 1,2,3,5,6, or 7 (Camhi, in Neural Control of Locomotion, R. Herman ed., 1976, in press). Results for typical examples of these six different giant axons are shown below. Cells 1 and 2 respond to wind from all angles. Cell 3 responds most strongly to wind from the front ipsilateral quadrant, and cell 5 from the rear ipsilateral quadrant. Cell 6 responds to wind from both anterior quadrants, and cell 7 from all directions so far tested except the rear contralateral quadrant. This directional information encoded by the giant fibers may allow the animal to direct its escape response with respect to a wind stimulus.

(Supported by NIH Grant NS-09083 to J.M.C.)

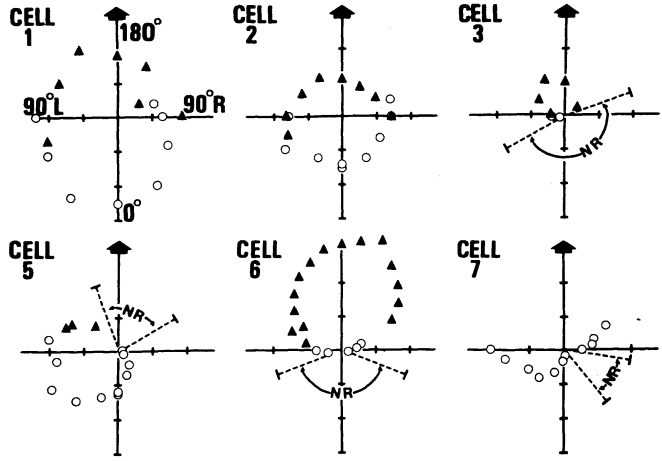
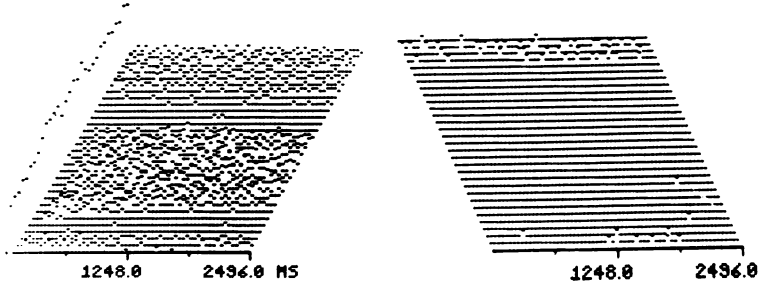


Figure: Polar plots of angle of wind stimulus vs. total number of action potentials evoked per stimulus (one division = 5 action potentials). In each graph, the origin coincides with the base of the cerci, and the arrow indicates the orientation of the insect. The wind delivery tube could be rotated from 90° left to 110° right. Circles represent responses to wind from these angles. Air drawn into the delivery tube was used to test the remaining 160° (solid triangles). Each point is the mean of five trials (standard deviations always less than ± 1). NR: range of angles where no action potentials were evoked. All recordings from left connective.

462 SPATIAL-TEMPORAL CHARACTERIZATION OF CRAYFISH CLAW EFFERENTS' FIRING PATTERNS DURING STEP MOVEMENTS OF THE DACTYLOPODITE. Bruce G. Lindsey and George L. Gerstein, Dept. of Physiology, Univ. of Penna., Philadelphia, Pennsylvania 19174.

The activities of excitatory and inhibitory motor neurons innervating the two antagonistic muscles of crayfish (Procambarus clarkii) claw have been monitored simultaneously during imposed opening and closing step movements of the dactylopodite. Individual PST histograms, each the sum of responses of a given neuron to the movement to and subsequent pause at a given position, have been stacked to generate a response plane, with time in the X axis, position in the Y axis, and probability of firing in the Z axis. The slow closer exciter (CE) response planes generated during opening steps reveal an early phasic response to each movement. The probability of later activity may vary as a function of both dactylopodite position and time. In many cases there is a zero probability of firing at some positions after the initial phasic response. During closing steps the phasic response is reduced or absent; later activity occurs with lower probability and may be more limited in spatial-temporal extent. The structure of opener inhibitor (OI) response planes is often qualitatively similar to CE planes, although the probability of firing is greater for both the phasic response and subsequent activity. OI may be active at positions where CE is not. Opener Exciter (OE) responses are less reproducible. OE may be silent except for occasional bursts; alternatively it may respond to step movements in both directions. A phasic response to closing steps, becoming more prominent for steps near the closed position (0 degrees), is most consistently observed. Limited observations of closer inhibitor (CI) activity reveal an early phasic response to steps in both directions; later activity varies in both space and time. Figures show examples of CE response planes to opening (left) and closing (right) steps over 18-36 degrees. Bottom histogram of each figure shows activity at 18 degrees. Stimulus parameters: 0.6 degrees/step; step duration 20 msec; interval between steps 2.5 seconds; 20 repetitions of stimulus series.



463 RECEPTORS ON CRUSTACEAN STOMATOGASTRIC CELLS. Eve Marder. Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris 75005 France.

The stomatogastric ganglion(STG) of Decapod Crustacea contains about 30 neurons, most of which make excitatory neuromuscular connections and inhibitory connections within the ganglionic neuropile(Maynard,1972, Ann. N.Y. Acad. Sci. 193: 59). Neurochemical and pharmacological findings allowed the assignment of ACh or glutamate as a transmitter candidate for each of the motor neurons in Panulirus interruptus. If one assumes that the same neurotransmitter is released by a neuron at both peripheral and central connections, one would expect that the central IPSP's evoked by the cholinergic motor neurons are mediated by ACh; on the basis of known synaptic connectivity at least 19 of the STG neurons should receive cholinergic inhibitory potentials. Iontophoresis of ACh onto the identified VD neuron which receives an IPSP from the PD neurons(which make cholinergic neuromuscular connections) elicited depolarizations. In similar experiments on unidentified STG cells in Cancer pagurus depolarizations were found in 40 cells from 15 preparations. Work is in progress to explore the possibility that different types of cholinergic receptors may be distributed at various synaptic and extrasynaptic regions. In contrast to the difficulties in finding ACh hyperpolarizations, iontophoresis of other putative transmitters onto identified Panulirus neurons elicited(at resting potential) depolarizing 5-HT responses; depolarizing, hyperpolarizing, and biphasic GABA responses; and hyperpolarizing glutamate responses.Supported by NIH 1F22NS00751 and the Helen Hay Whitney Foundation.

464 COMPARISON OF RHYTHMIC LEG MOVEMENTS IN THE COCKROACH: IMPLICATIONS FOR CENTRAL PATTERN GENERATORS. Stephen C. Reingold* (SPON: H. Howland). Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Walking in the cockroach Periplaneta americana L. involves rhythmic protraction and retraction of legs. Metathoracic legs step in alternation with each other. Abdominal grooming involves rhythmic protraction and retraction of a single metathoracic leg, which is raked across the abdomen to remove chemical or tactile irritants. Similarities in rhythmic movements of single metathoracic legs in the two behaviors were determined by motion picture analysis of behaving animals. Slight differences are seen in the position of leg segments and the excursion at leg joints in walking and grooming. These differences may reflect the different orientation of the whole leg during the two behaviors: during walking, the leg strikes the ground, while during grooming, the leg is lifted and placed in contact with the abdomen.

Further similarities in activity of individual metathoracic legs during walking and grooming were determined by electromyographic recordings from antagonistic muscles which control protraction and retraction at the coxal-femoral joint. Bursts of electrical activity from these muscles during walking and grooming show similar phasing between antagonists. Slight differences are seen in the burst durations of some muscles and in intervals between bursts of antagonists. Such differences may reflect differences in reafference during the two behaviors. Furthermore, experimental manipulation of sensory feedback from the moving leg during grooming can alter muscle burst duration, interburst interval, and phasing between antagonists. A third rhythmic leg movement--outward kicking used in righting behavior--is also similar to walking and grooming.

These data suggest that movement of single metathoracic legs during walking, grooming, and righting may be under the control of a common or shared central pattern generator. This central pattern generator may be subject to sensory input which determines details of muscle patterning used in the three behaviors.

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465 RESPONSES OF LEG MOTOR NEURONS TO ELECTRICAL STIMULATION OF GIANT AXONS IN THE COCKROACH PERIPLANETA AMERICANA. R. E. Ritzmann* and J.M. Camhi (SPON: A. Sastre). Section of Neurobiology and Behavior, Cornell Univ., Ithaca, N.Y. 14853.

The ventral nerve cord of the cockroach Periplaneta americana contains at least 14 individually identified giant axons which are excited by cercal wind-receptive hairs. Most of the giant axons respond preferentially to particular directions of wind, while others are omni-directional (Langberg, Westin and Camhi, present volume). Owing to these properties, the giant axons could mediate the directional evasive running evoked by wind puffs in this insect (Camhi et al., unpublished). Such a system offers the opportunity to study the role of numerous identified interneurons in a complex behavior. However, recent experiments have failed to demonstrate action potentials in leg nerves, or postsynaptic potentials in leg motor neurons, upon electrical stimulation of giant axons (Dagan and Parnas, J. exp. Biol. 52: 313-324, 1970; Iles, J. exp. Biol. 56: 647-656, 1972). The stimulus used in the above experiments was a current pulse just suprathreshold for action potentials in an undetermined number of giant axons.

We have performed similar experiments which demonstrate that stimulation of giant axons can result in excitation of leg motor neurons. We stimulated the ventral nerve cord with single current pulses just suprathreshold for evoking an action potential in an impaled giant axon. (The axon was subsequently identified by iontophoresis of procion yellow.) Such pulses, and often those of slightly lower amplitude, evoked depolarizing postsynaptic potentials (psp's) of up to 4 mV. from the cell body of motor neuron Df -- the fast coxal depressor -- and several unidentified motor neurons. However, intracellular stimulation of the impaled giant axon with single suprathreshold pulses evoked no discernible response in the motor neurons. In other experiments we verified, by extracellular recordings from the whole cord, that only giant axons appeared to be activated by whole cord stimuli which were sufficient to cause psp's in the motor neurons. For some motor neurons, more than one stimulus pulse was required to elicit a psp. This suggested that synchronous activity of several giant axons, or repetitive activity in one giant axon, is required to elicit motor neuronal activity. Indeed, natural stimulation by weak air currents (70 msec. duration, 3 m./sec. peak velocity) from any direction evokes trains of action potentials of about 200 to 700 Hz. in at least 6 giant axons (Langberg, Westin and Camhi, present volume and in preparation). We stimulated single giant axons intracellularly using pulse trains of 400 Hz. and 50-100 msec. duration -- parameters we deemed necessary in view of the absence of accompanying activity in other giant axons. Such stimuli evoked action potentials in metathoracic nerves 6Br4 and 5r1 containing, respectively, coxal levator and depressor motor axons. We are presently repeating these experiments, using intracellular recording in motor neurons to determine whether subthreshold events occur with shorter trains of stimulation, and to define which motor neurons are excited by which giant axons.

Our results establish that motor outputs to the legs can result from patterns of activity in giant axons similar to those which can be evoked by gentle wind stimulation.

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466 TEMPORAL PATTERNING IN SPIKE TRAINS FROM AN INSECT MOTOR-NEURON. J. M. Sidie and G. Hoyle. Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

The anterior coxal adductor muscle (AAdC) of the locust Schistocerca gregaria is innervated by a single 'slow' excitor neuron that fires tonically ($\bar{X}$ = 8-28 Hz). Its spike trains were analyzed using serial correlation coefficients (S. C. C.). There is a strong probability for short interspike intervals to be followed by long intervals and vice versa, but there is no correlation with subsequent intervals. S. C. C. magnitude, and therefore the degree of temporal structuring of the spike train, increases with increasing frequency. The threshold for significant coefficients is approximately 10-15 Hz and it can be shown statistically that there is underlying negative correlation. This feature, numerous spike doublets (interspike intervals 15 ms or less), is the single conspicuous feature of the temporal structure of the spike train.

The neuron contains a pacemaker region that tends to even out the effects of randomly-timed inputs, but either as a corollary, or independently, the impulses tend to be generated in pairs. These show up in a joint interval histogram (Fig.) as two equal clusters, long followed by short (LS), and short followed by long (SL), intervals. The pattern is functionally relevant, because fine-tuned control of tension is achieved by such temporal patterning. At any given frequency, as the amount of negative serial correlation in the trains is increased, tension in the muscle is enhanced in a non-linear fashion. The amount of this tension increment is greatest at higher frequencies (40-60 Hz).

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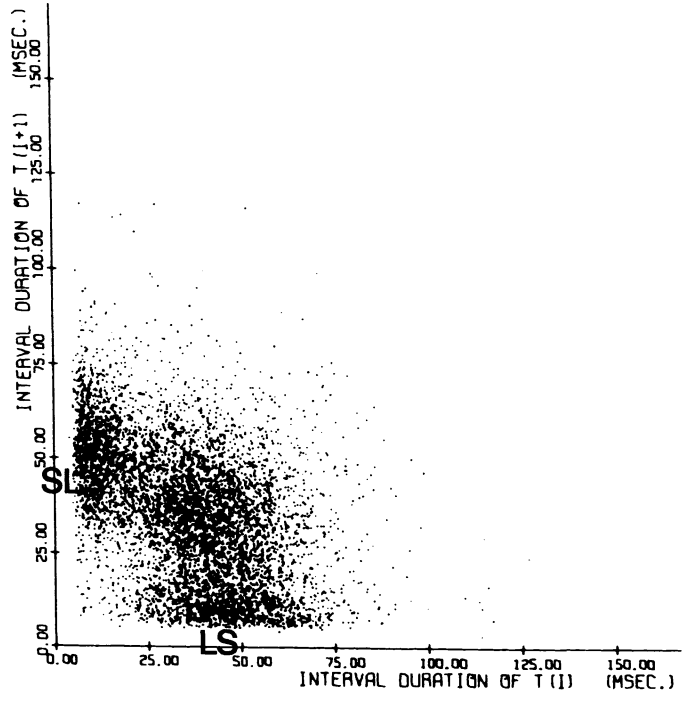


467 ENDOGENOUS LEVELS OF OCTOPAMINE, SEROTONIN, DOPAMINE AND ACETYLCHOLINE IN SPINY LOBSTER PERICARDIAL ORGANS. Robert E. Sullivan*, Brenda Friend* and Richard McCaman. (SPON: E. A. Maynard) Div. Neurosci., City of Hope Medical Center, Duarte, CA 91010.

A segmental nerve (SN) exits dorsally from each thoracic ganglion in macrurans and from homologous regions of the brachyuran thoracic mass. The SN's travel to the pericardial cavity where their inosculations are known as pericardial organs (PO's). Brachyuran PO's contain peripheral cell bodies and several kinds of neurosecretory processes. D. Maynard (Gen. and Comp. Endocrin.,1:237,1961) considered the PO to be one terminus of a larger neurosecretory system including the anterior ramifications of SN 1. Analogous systems of median connectives and dorsal trunks occur in stomatopods, ostracods and isopods. The discovery of octopaminergic cell bodies in macruran SN's has led to the hypothesis that a primitive analogue of the vertebrate sympathetic nervous system is present in crustaceans (Wallace et al., Brain Res.,74:349,1974).

In the PO of the spiny lobster, Panulirus interruptus, segmental nerves contribute processes to three pairs of ligamental nerve plexuses (LNP's) terminating at the entrances to heart ostia where the cardiac ligaments insert (Fig. 1). SN's 1, 2 & 3 anastomose in the rostrolateral pericardial cavity to form the lateral pericardial plexuses (LPP); nerve trunks arising from LPP's ramify extensively as the anterior and posterior ligamental nerve plexuses (ALP and PLP). The medial ligamental nerve plexuses (MLP) arise from a separate, fourth nerve trunk, tentatively designated as SN 4. At least four classes of neurosecretory processes can be distinguished in the Panulirus LNP on the basis of differing vesicle morphology; small processes abutting the acellular epineurium exhibit morphological indications of release sites, but no conventional synapses have been observed. Octopamine (OCT), dopamine (DA) and serotonin (5-HT) are synthesized in the ligamental nerve plexuses (Sullivan and Friend, in prep.) and in the crab PO (Barker and Hooper, Neurosci. Abs.,1:395,1975). We have proposed that PO monoamines, either individually or in concert, modulate the activity of the cardiac network.

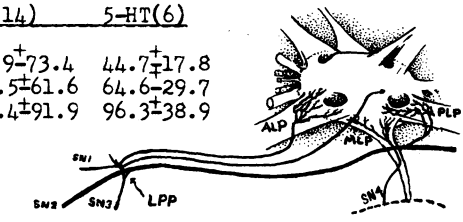
A neurosecretory modulator may have to be released in substantial quantity in order to attain an effective blood concentration. We have determined the endogenous levels of OCT, DA, 5-HT and acetylcholine (ACh) in Panulirus PO structures using sensitive radiochemical enzyme methods. Previously, 5-HT was thought to be the major biogenic amine in decapod PO's. However, we find that the amount of OCT is at least three times that of 5-HT (Table 1). The total number of pM of each compound in the lobster PO's amounts to: OCT=1221±322; 5-HT=411±120; DA=102±15;ACh=88±14. Histamine levels were at least an order of magnitude less than ACh levels. Due to the complicated neuroanatomy, diffusion parameters and hemolymph bulk flow, it is difficult to predict the 5-HT and OCT concentration gradients that would obtain when LNP's are stimulated in vivo. However the data place an upper limit on the amount of monoamines that could be released by the PO and should help in designing physiological experiments to test the modulation hypothesis.

Acetylcholine-containing neurons may be involved in sensory functions and/or in regulating the release of monoamines. A number of small, bipolar nerve cell bodies are located in the ligamental nerves to the PLP's which contain the highest levels of ACh.

COMPOUND	ACh(8)	DA(6)	OCT(14)	5-HT(6)
TISSUE	picomoles/tissue sample			
ALP	7.0±1.9	10.9±3.9	118.9±73.4	44.7±17.8
MLP	7.0±2.6	14.0±4.8	197.5±61.6	64.6±29.7
PLP	30.0±11.3	26.1±2.7	253.4±91.9	96.3±38.9

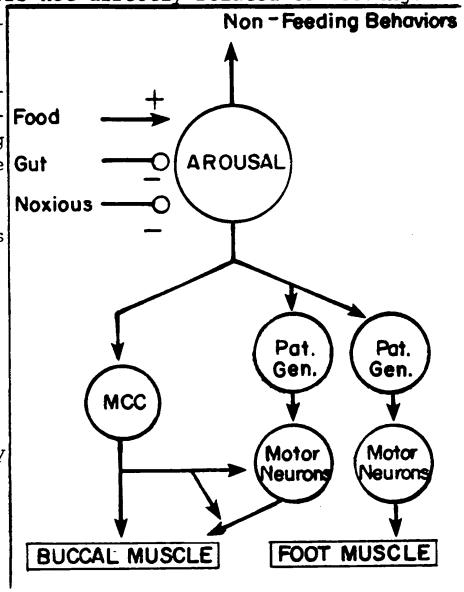
TABLE 1.

fig.1



468 AROUSAL OF FEEDING BEHAVIOR OF APLYSIA. A. Susswein*, I. Kupfermann and K.R. Weiss, Dept. Psychiat., Coll. of Phys. & Surg., Columbia U., and N. Y. State Psychiat. Inst., New York, N.Y. 10032.

Exposure of Aplysia to food produces relatively long-lasting alterations of a number of behaviors. We suggest that these alterations are due to the operation of a central arousal state. Arousal due to food stimuli is reflected in changes in feeding behaviors (priming) as well as in more generalized effects on non-food-related responses (Advokat et al., this meeting). The priming component of arousal affects both appetitive (orienting) and consummatory (biting) behaviors. The present study has concentrated on 2 measures of the consummatory phase of feeding: latency and amplitude or strength (sensitizing component) of feeding responses. When non primed animals are tonically stimulated with food, amplitude of successive feeding responses progressively and rapidly increases and latency decreases. Removal of seaweed results in an initially rapid decay, followed by a slow decay of priming. Both rise and decay of priming are modulated by variables which, in previous studies, were shown to affect intensity of feeding movements. Thus, the rate of rise and decay of priming is a function of the satiation level and the concentration of the food stimulus. The effects of satiation on priming are mimicked by feeding animals with non-nutritive bulk, indicating that post-ingestion bulk stimuli are responsible for this effect. The metacerebral cell (MCC), a large serotonergic neuron, is known to increase contractility of muscles producing feeding; thus, activity of the MCC could mediate the effects of priming on amplitude. To test this hypothesis, we monitored (extracellularly) the activity of the MCC in freely-moving animals. The MCC was silent in non-primed animals. Brief exposure of the animal to food resulted in firing of the MCC (1-10/sec); firing was maintained as long as the animal exhibited behavioral signs of arousal. Recordings from the MCC in acute preparations indicate that this neuron is not endogenously active. Therefore it seems likely that persistent activity following food stimuli is due to tonic excitatory input from a higher-order arousal system. Fig. 1 illustrates one way of conceptualizing the operation of the food arousal system in Aplysia. Food stimuli elicit (and gut and noxious stimuli inhibit) persistent activity in an arousal system. The arousal system has two main outputs, a priming output that affects food related behaviors, and a non-priming output that affects behaviors not directly related to feeding. The priming output from the arousal system in turn expresses itself through two pathways. One pathway impinges on various pattern generators (e.g. those generating searching movements, and those generating biting responses) and increases the probability that these generators can become active. A second pathway from the arousal system results in tonic firing of the MCC, which produces an enhancement of the intensity parameters of the biting response. As suggested by previous studies (Weiss, Cohen and Kupfermann, 1975; Weiss, Schonberg, Cohen, Mandelbaum and Kupfermann, this meeting) the MCC effects may be exerted at 3 sites: 1) centrally at motoneurons, 2) at motoneuron terminals, and 3) directly on the muscle.



469 TWO DIFFERENT AND COMPATIBLE INTRACELLULAR LABELS: A PRELIMINARY STRUCTURAL STUDY OF IDENTIFIED SENSORY AND MOTOR NEURONS WHICH MEDIATE THE GILL WITHDRAWAL REFLEX IN APLYSIA CALIFORNICA. E. B. Thompson, C. H. Bailey*, V. F. Castellucci, and E. R. Kandel. Div. Neurobiology and Behavior, Col. P & S, Columbia University, New York, N. Y., 10032.

Ultrastructural analysis of the synaptic contacts which mediate behavioral plasticity of the gill-withdrawal reflex requires the use of special labeling techniques capable of revealing the fine processes and synapses of the identified neurons which are components of the neural circuit for this behavior. Ultimately it will be necessary to identify all elements which participate in a specific synaptic contact. This objective can be partially attained by separately studying the morphology of the individual neurons involved. An unequivocal identification of such a synaptic connection would result from the simultaneous use of separate labels in the synaptically related neurons. In the accompanying abstract we have described the general morphology of the terminals of identified sensory neurons labeled by intracellular injection of horseradish peroxidase (HRP). Here we describe studies of the motor neuron L7, which receives synapses from these sensory neurons. For these preliminary studies we have alternated, in different ganglia, radioautography after intrasomatic injection of tritiated sugar precursors of glycoproteins (Thompson et al., Soc. Neurosci., 5th Ann. Meet., 1973) with intrasomatic injection of HRP (Muller and McMahan, Anat. Rec., 1975), thus exploiting the advantages of each labeling technique.

In some preparations we have successfully combined the HRP method with the radioautographic method by injecting sensory neurons with HRP and cell L7 with ^{3}H-N-acetyl-D-galactosamine. Radioautography of sections from doubly injected preparations reveals silver grains over the soma and processes of L7, while HRP is present in the soma, processes and terminals of the sensory neurons. Complete serial sampling of 4 μm sections with the light microscope reveals areas of potential contact between the injected neurons. By re-embedding and thin sectioning for electron microscopy, we now hope to use this technique to study double labeled synapses.

470 MODULATION OF MUSCLE CONTRACTION BY A SEROTONERGIC NEURON: POSSIBLE ROLE OF CYCLIC AMP. K.R. Weiss, M. Schonberg*, J. Cohen*, D. Mandelbaum*, and I. Kupfermann, Depts. of Neurol. and Psychiat., Coll. of Phys. and Surg., Columbia U. and N.Y. State Psychiat. Inst., New York, N.Y. 10032

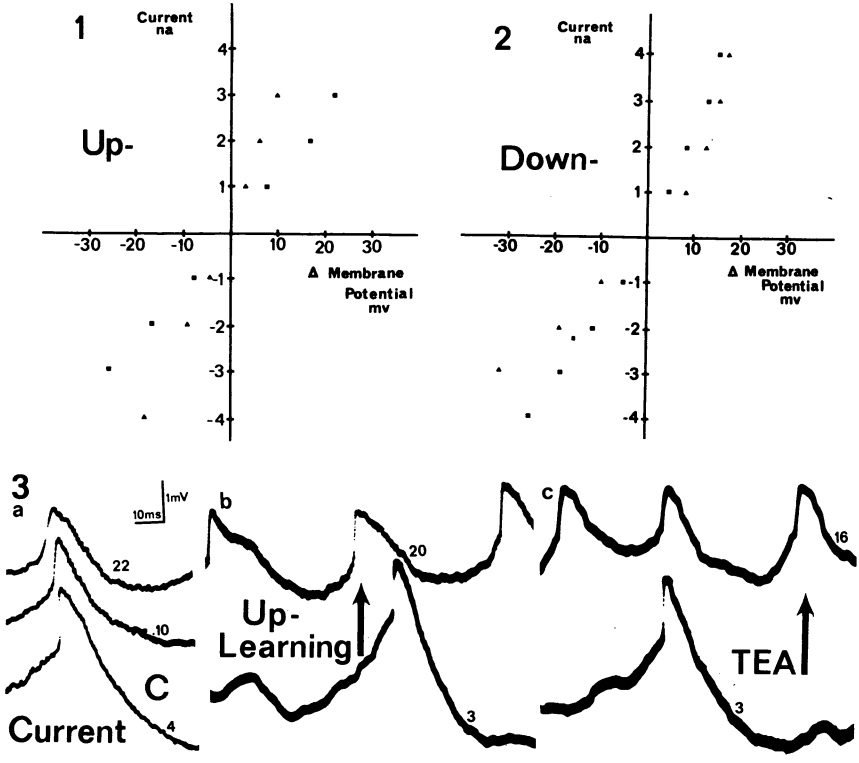
Previous experiments have suggested that the two symmetrical metacerebral cells (MCCs) of Aplysia are involved in some aspect of food arousal. It was shown that firing of the MCC produces a central excitatory effect on buccal motoneurons, and in addition, has a peripheral action that results in a long-lasting increase of buccal muscle contractions elicited by uniform bursts of motoneuron spikes (Weiss, Cohen and Kupfermann, 1975). In the present experiments we have attempted to analyze the mechanism of the peripheral potentiation. The data suggest that potentiation results from two different actions of the MCC at the muscle, 1) enhancement of the size of EJPs and 2) a direct effect on muscle contractility. Stimulation of the MCC produces a small, but distinct, increase in the size of motoneuron EJPs with no change of the decay constant. These data suggest that serotonin, the transmitter of the MCC, may mediate a form of heterosynaptic facilitation at the muscle, as has been previously suggested for central synapses in the abdominal and pleural ganglia (Brunelli, Castellucci and Kandel, 1975; Shimahara and Tauc, 1975). The time course of the build-up and decay of the enhancement of EJP size was markedly different in different motoneurons innervating the same muscle. In motoneuron ARC 3 the EJP enhancement and muscle potentiation had markedly different time courses, suggesting that for this neuron, MCC potentiation of contraction involves an effect on muscle contractility, as well as facilitation of the EJP. In motoneuron ARC 2, EJP facilitation and muscle potentiation had a parallel time course. However, it was possible to show that the MCC could potentiate contractions elicited by ARC 2 even when the EJP was experimentally reduced in size below control levels. Therefore, although in ARC motoneuron 2, EJP enhancement and muscle potentiation are normally parallel, the muscle potentiation, in part, appears to be due to a direct effect of the MCC on contraction coupling. We have explored the possibility that the MCC may produce its actions by activating the synthesis of cyclic AMP. Serotonin applied to an intact buccal muscle, or a cell-free homogenate of muscle, increased the synthesis of cAMP. In addition, incubation of the muscles in analogs of cAMP (8BT-cAMP, 8PCPT-cAMP) simulated the action of the MCCs. This enhancement of muscle contraction occurred in spite of the fact that these compounds reduced the size of the EJPs (perhaps by an anticholinergic action). This dissociation of the action of the analogs of cyclic AMP on muscle contraction and EJP size indicated that these drugs enhanced the contractions of the muscles by directly modulating their contractility.

Converging evidence from both vertebrate and invertebrate studies indicates that biogenic amines may be involved in modulation of neural and muscular excitability and that this action may be mediated by cyclic AMP. The present data support the notion that the increased intensity of biting responses seen during arousal of feeding behavior in Aplysia may be mediated by the MCC and that the MCC actions may be related to enhanced levels of cAMP due to the action of serotonin. Evidence obtained by others in the abdominal ganglion suggests that a similar mechanism may underlie increased gill contractions following a noxious sensitizing stimulus (Brunelli et al., 1975). Our results, taken together with those of Kandel and associates suggest that there may be multiple expressions of an arousal system or systems in Aplysia, involving anatomically distinct neuronal structures. Nevertheless, arousal or sensitization of different response systems may utilize biochemically similar mechanisms. Indeed, these results encourage speculation that certain aspects of arousal in vertebrates may also be related to the ability of the biogenic amines to stimulate cAMP and thereby gain access to metabolic processes in cells.

471 MEMBRANE RESISTANCE CHANGES ASSOCIATED WITH SINGLE, IDENTIFIED NEURON LEARNING. M. H. Woollacott and G. Hoyle, Biology Department, University of Oregon, Eugene, Oregon 97403.

The locust anterior coxal adductor motor neuron was trained by computer to fire at higher (up-learning) or lower (down-learning) frequencies. Its input resistance before and after learning was determined using a double-barreled soma electrode. Current/voltage plots were linear in the frequency range 0-20 Hz. The resistance shifted during learning. Examples are shown in Fig. 1. The resistance before up-learning was 4 M at 12.5 ± 1.3 Hz (Fig. 1, triangles). After a single step to 17.2 ± 0.9 Hz, the resistance increased to 7 M (Fig. 1, squares). The initial resistance in down-learning was 9 M at 20.0 ± 1.1 Hz (Fig. 2, triangles). In a single period it fell to 12.1 ± 2.2 Hz, and the resistance dropped to 4 M (Fig. 2, squares).

A change in shape of the soma-recorded action potential accompanies frequency changes (Fig. 3a: numbers = mean frequencies). At lower frequencies there is a large undershoot, suggesting membrane potential-activated potassium conductance that is greatly reduced at higher frequencies. Similar changes in shape accompany learning (Fig. 3b). Injection into the neuron of the K^+-blocking agent TEA eliminated the undershoot, accompanied by increased frequency (Fig. 3c). It is therefore postulated that the learning steps may be associated with shifts in gK. These are: decrease in gK and increased resistance in up-learning; increase in gK and decreased resistance in down-learning. The conductance changes in turn cause the frequency changes. This research was supported by NSF Research Grant BNS75-00463 and by the Sloan Foundation.



472 THE CEREBRAL GANGLION COUPLES THE LEFT AND RIGHT ELECTRORETINOGRAM CIRCADIAN RHYTHMS IN CRAYFISH PROCAMBARUS BOUVIERI (O). Baltazar Barrera Mera and Esperanza M. Abasta* Depto. de Fisiología, Fac. de Medicina, Univ. Nacl. de México, México 20, D.F.

Bilateral electroretinographic (ERG) circadian rhythm was obtained in intact and in "splitbrain" crayfish. The experiments were performed under continous darkness condition interrupted only by brief test light stimuli (0.09 ft^2 candle). The lenght of the period, the $\alpha : \rho$ ratio, and the phase showed essentially the same values in both eyes in the intact animal. The application of light for 60-minutes (0.125 ft^2 Cd) at either eyestalk induced a short lasting bilateral decrement in ERG voltage and a similar phase shift in both left and right ERG rhythms. In a similar way the application of 6-hydroxidopamine (6-OHDA) in one eyestalk produced an infradian rhythm (τ = 28-36 hrs) in both eyes. After the bisection of the cerebral ganglion, the phase angle; the $\alpha : \rho$ ratio, and the range values were modified as compared with the controls. In this condition the injection of 6-OHDA induced only an ipsilateral ERG infradian rhythm without changing the contralateral ERG circadian oscillation.

The above mentioned changes suggest an important role of the cerebral ganglion in the coupling mechanisms between both "pacemakers" of left and right ERG circadian oscillations.

473 Reduction of rat attack on colony intruders after septal damage. Robert J. Blanchard, Dept. of Psychology, University of Hawaii, Honolulu, Hawaii 96822.

Dominant male rats (alphas) of established rat colonies show a clear pattern of biting attack on intruders into their colonies. Electrolytic lesions of the septal area were made in such alpha males, producing major damage to the medial septal nucleus and lesser damage to the lateral septal nuclei, nucleus of the diagonal band, and pre-commissural fornix. In comparison to operated control alphas, rats with septal damage showed a drastically reduced level of attack on colony intruders on tests made 2 and 9 days after surgery. By 23 days post-surgery, however, control-experimental differences had disappeared or were substantially reduced.

Septal-damaged alpha males showed reduced durations of piloerection, lateral attack and boxing to intruders, and fewer instances of biting. Moreover, strangers confronted by these rats were much less often wounded than were animals placed in control colonies, or in the colonies of the experimental animals prior to surgery. Some, but not all, of the septal-damaged rats showed irritability to handling. However, there was no reliable correlation between such irritability and intraspecific attack tendencies for these animals.

474 QUANTAL ANALYSIS IN EXCITATORY FAST FLEXOR NEUROMUSCULAR SYNAPSES OF THE CRAYFISH. Thomas H. Brown* and Nicki A. Newby (SPON: D. H. Perkel). Dept. of Biol. Sci., Stanford University, Stanford, Ca. 94305.

The crayfish fast abdominal flexor muscles are innervated by a giant motor neuron, which displays low-frequency synaptic depression (LFD), and several nongiant motor neurons, which show no LFD (Bruner & Kennedy, 1970, Science 169: 92). Physiological analysis of these synapses has been limited by the inability to resolve single quanta above the background noise. We have investigated the properties of these neuromuscular junctions during normal development, with the hope of finding a stage at which quanta are readily observed and quantal analysis would be feasible. In mature adults we find that the muscle "fibers" can be quite large in diameter, each consisting of many "subunits" electrically interconnected by sarcoplasmic bridges. The input resistance (R_{in}) is thus extremely low (10 to 40KΩ), which explains the inability to resolve single quanta. In animals less than 2 cm in length the "fibers" are smaller in diameter, consist of fewer "subunits," and R_{in} is an order of magnitude higher. Small spontaneous depolarizing potentials, which are not abolished by nerve section or bath-applied tetrodotoxin, are clearly identifiable in these muscles. The timing of their appearance can be approximated by a Poisson process. These miniature potentials form a single population with respect to amplitude, with a mean value ($\bar{v}_1$) typically less than 100μV in 1 cm animals. The excitatory junctional potentials (ejps) evoked by stimulating single motor axons are generally large (>20mV) even in very young animals. In these animals direct estimates of the mean quantal content ($m = \bar{ejp}/\bar{v}_1$) indicate that $m > 100$. The coefficient of variation method ($m = 1/CV^2$) yields similarly high m values, and further indicates that $m > 1000$ in mature adults. We conclude that quantal analysis can be performed at these synapses and that m is much larger than previously reported at other arthropod neuromuscular junctions.

475 GRADED ACTION POTENTIALS IN APLYSIA NEURONS. H. Bryant and E. Decima. Dept. Anat., Sch. Med., UCLA, Los Angeles, CA. 90024.

Experiments have been performed on identified somata and axons of Aplysia abdominal ganglion neurons which clearly demonstrate the existence of decremental conduction. The classical concept of decremental conduction holds that under certain experimental conditions: 1) the amplitude of the nerve impulse is graded and depends on the magnitude of the stimulus which evoked it; 2) the distance through which the impulse conducts depends on its size; and 3) both the impulse magnitude and its speed of conduction can change incrementally or decrementally during propagation depending on the state of the nerve fiber. All of these classical concepts have been confirmed using a combination of intra- and extracellular recording and stimulating techniques in single somata and fibers under a variety of experimental conditions (e.g., Na^+ deprivation, local anesthetics, high K^+ concentrations). For example, in a Na^+-free medium, somata of the abdominal ganglion produce action potentials whose amplitudes are graded with the stimulus strength. The relationship between stimulus strength and impulse magnitude is not constant but changes with time of exposure to the Na^+-free medium. In a Na^+-free or high K^+ (10 times normal) medium or in the presence of 2-10 mM xylocaine, the axon of the giant cell (R2) also produces an impulse whose magnitude depends on the stimulus strength. Action potentials produced at the stimulating cathode may conduct either incrementally or decrementally depending on the severity of depression of the nerve. A cathodal DC bias and a stimulating tetanus tend to relieve decremental conduction produced by Na^+-deprivation, whereas an anodal bias relieves decrement produced by a high K^+ medium. The significance of the ability of nerves to conduct decrementally (or incrementally) on the interpretation of neurophysiological observations and on the integrative processes in the nervous system will be discussed.

476 FUNCTIONAL VIABILITY OF A CNS DENDRITIC ARBORIZATION SEVERED FROM SOMA. Richard D. Clark* (SPON: M. J. Cohen). Dept. Zoology, UC Berkeley, CA. 94720.

Midline hemisection of the metathoracic ganglion of crickets was performed to separate an identified motor neuron, the contralateral dorsal longitudinal motor neuron (CDLM), into proximal and distal segments. The proximal segment, confined to the ganglion, was composed of the CDLM soma and initial neurite. The distal segment comprised the bulk of the CDLM dendritic arborization within the ganglion, and the axon in the peripheral motor nerve trunk. Function of the distal segment was monitored from 0-168 days after separation from the soma, by extracellular recording from the muscle bundle innervated by CDLM. Cobalt dye-fills of the dendritic arborization and axon were performed following physiological tests. Axonal conduction in the CDLM distal segment in the peripheral nerve was preserved as long as some morphological remnant of the distal segment was present; in most cases, the isolated dendritic arborization and peripheral axon survived structurally up to 100 days after separation from the soma. In the extreme, axonal conduction persisted to 168 days after soma section. This period of survival is within the range of extreme survival time reported for axonal segments in the distal stump of the crayfish claw opener nerve. Neuron-muscle transmission and miniature end-plate potentials in the muscle bundle innervated by the axon of the distal segment appeared normal up to 45 days postoperative, at which point a naturally-occurring breakdown in the muscle made further measurements impossible. The isolated dendritic arborization could be excited by normal pre-synaptic input up to 72 days after cutting away the soma. (Supp. by PHS NS09074 & 5 TO1 GNO 1021)

477 CHRONICALLY RECORDED ACTIVITY OF THREE IDENTIFIED GIANT NEURONS IN FREELY-BEHAVING APLYSIA. J.S. Cobbs* and H.M. Pinsker (SPON: D. Eaton). Marine Biomed. Inst., Univ. of Texas Med. Br., Galveston, Texas 77550.

Three identified giant neurons of unknown behavioral function have cell bodies in the abdominal ganglion and axons in the right (R1, R2) and left (L1) pleurovisceral connectives of Aplysia. Extracellular spikes were recorded chronically with cuff electrodes and could be identified since R1 has a lower threshold for extracellular stimulation, smaller action potentials, and a higher conduction velocity than R2. Threshold, amplitude, and conduction velocity of L1 are similar to those of R1. R1 and R2 project to the parapodia through the same peripheral nerves (Feinstein, personal communication). Since high frequency stimulation of R1 alone, or R1 and R2 together, does not produce any observable behavior in the intact animal, these cells are probably not command or motor neurons. These normally silent cells can be activated by novel, sudden-onset, mechanical stimulation, but their long latencies and irregular responses suggest they are not first-order sensory neurons. R1 and L1 fire more spikes and decrement less with repeated mechanical stimulation than R2. R1, L1 and R2 receive synaptic excitation from stimulation of peripheral nerves of the abdominal ganglion (Frazier et al., 1967). In the intact animal R2 spikes are initiated in the neuropil of the abdominal ganglion and conduct rostrally in the connective away from the cell body (c.f., in vitro work of Hughes & Tauc, 1963). However, the spikes of R1 and L1 are initiated rostrally and conduct caudally in the connective toward the cell body. We are examining the hypotheses that (1) these neurons propagate action potentials bidirectionally, and (2) that they have a modulatory (Weiss, Cohen & Kupfermann, 1975) rather than a command, function. Supported by NIH grants NS 11255 and NS 12223.

478 THEORY OF SPATIALLY SYNCHRONIZED OSCILLATORY RESPONSES IN THE LIMULUS RETINA. Bernard D. Coleman, Carnegie-Mellon University, Pittsburgh, Pa. 15213, and George H. Renninger, University of Guelph, Ontario N1G 2W1.

Available experimental data on the dynamic properties of lateral and self-inhibition in the Limulus retina indicate that the rate $r_k(t)$ of firing of an ommatidium (i.e., the k'th) located near the center of a region subjected to a spatially uniform excitation E(t) should obey, to a good approximation, an integral equation of the form,

$$r_k(t) = m\left[E(t) - \frac{K_S}{\delta_S}\int_0^\infty e^{-s/\delta_S} r_k(t-s)ds - \frac{K_L}{\delta_L}\int_0^\infty e^{-s/\delta_L} r_k(t-\tau-s)ds\right]. \quad (1)$$

Here, $m(x) = \max(0,x)$; δ_S is the decay time for self-inhibition; δ_L is the decay time for lateral inhibition; τ is the delay in lateral inhibition; K_S is a dimensionless parameter measuring the strength of self-inhibition; and $K_L = \Sigma_i \kappa_{ki}$, where the κ_{ki} are (non-negative) dynamical inhibitory coefficients, and Σ_i indicates a sum over all ommatidia, (other than the k'th) which lie in the illuminated region. The number K_L increases with the size of the illuminated region. When K_L exceeds a critical value K_L^*, the stable solution r_k of (1) for $E(t) \equiv E^\circ = \text{const.}$ is non-constant and periodic; this solution describes a response occurring in spatially synchronized bursts. For $K_L < K_L^*$, the stable solution for $E(t) \equiv E^\circ$ is of the form $r_k(t) \equiv r^\circ = \text{const.}$ We here report the results of numerical studies of the effect which abrupt changes in E have upon solutions of (1) for K_L below but near K_L^*, and we show that the form of such solutions is very sensitive to the occurrence of small sudden increases or decreases in excitation.

479 NEUROMUSCULAR ORGANIZATION IN PLEUROBRANCHAEA. George H. Dersham and Richard M. Lee. Edsel Ford Institute, Detroit, MI. 48202.

The organization and control of movements associated with feeding behavior in Pleurobranchaea was investigated by recording from central neurons and peripheral musculature in a modified (restrained) whole-body preparation. Ablation of some or all of the CNS was done in some cases to demonstrate peripheral influences. Most of the muscles involved in proboscis eversion are polyneuronally innervated and many show marked facilitation to stimulation frequencies as low as 2/sec. Direct and reflex stimulation of motor pathways showed that most muscles receive direct, short-latency input from central motorneurons plus excitation probably mediated by a peripheral nerve net. Spikes assumed to be nerve net mediated have longer latencies, are larger, and predominate in some feeding movements. Direct innervation pathways mediate small twitch contractions which relax slowly; repeated stimulation may yield sustained contractions at relatively low frequencies.

During forceful buccal mass protractions, there is marked synchrony of firing in several muscles which do not show common innervation from the CNS. Similar responses to direct mechanical stimulation (stretch or prodding) of the muscles also occur.

It is suggested that: (1) central and nerve net innervation involve parallel but interacting pathways; (2) some coordinated muscle activity may be peripherally organized via the nerve net. Other alternatives to (2) such as non-neural (irritability) responses are also being investigated.

480 BAG CELL ACTIVITY AND EGG-LAYING IN FREELY-BEHAVING APLYSIA. F.E. Dudek and H.M. Pinsker, Dept. of Zoology, Univ. of Toronto, Toronto, Ontario and Marine Biomedical Inst., Univ. of Texas Med. Br., Galveston, TX 77550.

Two bilaterally symmetrical clusters of neuroendocrine bag cells, located in the abdominal ganglion of Aplysia at the base of the pleurovisceral connectives, are believed to mediate egg-laying by release of a peptide hormone(s). In vitro intracellular studies showed that strong stimulus trains to either connective could cause a synchronous bag cell after-discharge (Kupfermann & Kandel, 1970) and release of a hormone(s) into the perfusate that induced egg-laying when injected into another Aplysia (Kupfermann, 1970). However, it has not been demonstrated that egg-laying can only occur if preceded by bag cell activity. During an after-discharge, the long-lasting compound action potentials could be recorded extracellularly from a pleurovisceral connective and were usually propagated from distal neurites toward the somata (Dudek & Blankenship, 1975). With extracellular cuff electrodes in freely-moving Aplysia, we have examined bag cell activity evoked by brief, intense trains of electrical stimuli. Bag cell spikes were identified by their long duration and irregular waveform. The direction of propagation and the temporal pattern of action potentials during the in vivo after-discharge were similar to the in vitro preparation.. Furthermore, the effects of these electrically evoked after-discharges were similar to the injections of bag cell extracts; that is, they were often, but not always, followed by egg-laying (Strumwasser, Jacklet & Alvarez, 1969). In future studies with the chronic monitor, we shall determine if repetitive bag cell action potentials are necessary for natural episodes of egg-laying. Supported by NIH grants NS 11255 and NS 12223.

481 VOLUME REGULATION IN SNAIL NEURONS. Barton B. Dunning* and Xenia Machne. Dept. Pharmacol., Sch. Med., Univ. Minn., Minneapolis, MN 55455; *Univ. Neb. Med. Center, Omaha, NE 68105.

The spheroidal neurons of the isolated subesophageal ganglionic mass of the land snail Helix aspersa were examined for their response to osmotic challenge. The osmotic stimulus was produced by altering the extracellular concentration of NaCl. Cell diameter was measured using an image splitting eyepiece (Vickers Inst., York, England) at 100X to an accuracy of 1-2%. Cell volume was calculated assuming that the monopolar neurons examined were spherical. Changing the snail Ringer bath from isosmotic to hyposmotic resulted in a swelling of the cells with peak volume being reached in 10-20 min; then the swelling began to decrease spontaneously, with the cell volume usually returning to normal within 1 hr. Measurements of membrane resting potential, action potential height and membrane resting resistance made at this time were little changed from control values. Returning the preparation to the isosmotic solution lead to an undershoot of cell volume below the control value. This volume regulatory response in hypotonic solutions is reversibly blocked by DNP. In contrast, hyperosmotic solutions resulted in cell shrinkage with no spontaneous reversal. Returning the preparation to the isosmotic solution usually resulted in a gradual return of cell volume to the original size. This indicates there is little or no volume regulation by these cells in hypertonic solutions. In addition to regulating volume in hypotonic solutions, it appears that these cells also maintain a roughly normal transmembrane ionic distribution during the regulatory response leading to the conclusion that the cells remain viable from the electrical point of view in hypotonic solutions.

482 OSCILLATORY NEURAL NETWORK GENERATING LEECH SWIMMING MOVEMENT.
W. O. Friesen, M. L. Poon* and G. S. Stent. Dept. Mol. Biol. University of California, Berkeley, California 94720

The swimming undulations of the leech Hirudo medicinalis are the result of metachronal antiphasic contractions of segmental longitudinal muscles in the dorsal and ventral body wall. The segmental motor neurons innervating these muscles can maintain their coordinated impulse burst rhythm in an isolated nerve cord preparation, indicating that the swimming movement is generated by a central neural oscillator. We have found that the motor neuron impulse burst rhythm arises from synaptic inputs provided by a set of four rhythmically active interneurons present in each segmental ganglion. These interneurons form part of the central neural oscillator because passage of current into one of them can shift the phase of the entire impulse burst rhythm. The interneuron set is interconnected by inhibitory synapses and the resulting neural network has oscillatory properties that account for the observed activity rhythm. Electrophysiological and anatomical evidence indicates that the axons of three of the interneurons project to anterior ganglia and that the axon of the fourth interneuron projects to posterior ganglia. It is envisaged that the posterior projection maintains the front to rear phase progression of the segmental impulse burst rhythms responsible for the metachronal wave and that the anterior projection maintains the period of the rhythm.

483 CHANGES IN INTERSPIKE INTERVAL IN FIBERS USING A TEMPORAL SEQUENCE CODE.
Stephen A. George, Peter Rugg*, and David Mastronarde* Neuroscience Program, Amherst College, Amherst, MA 01002

In several nerve fiber preparations, the intervals between nerve impulses in an impulse train have been shown to change as that train propagates along the fiber. However, it is not known whether these changes in temporal sequence affect the transmission of information in the fiber. Such an effect could occur only in fibers that make use of the temporal sequences of impulses, as distinct from the average impulse frequency, to encode information. Therefore, we looked for changes in temporal sequence in spike trains, elicited by electrical stimulation, in fibers of the visceropleural connectives in Aplysia, which are known to use a temporal sequence code (Segundo et al., J. Exp. Biol. 40: 643-667, 1963).

Substantial changes in the temporal sequences of impulses were observed in this preparation. When spikes were initiated between 15 and 60 msec apart, the interval between them decreased during propagation such that, for example, spikes initiated 30 msec apart at one end of a connective would arrive at the distal end 25.5 msec apart. In contrast, when initial intervals were less than 15 msec or more than 60 msec, the intervals increased during propagation. These changes in interval can be accounted for by effects on spike conduction velocity during refractory, supernormal, and long-term depression periods following the first impulse.

Thus temporal sequences of nerve impulses are not maintained during propagation even in this preparation in which information is encoded in the temporal sequences. Whether these changes in sequence represent noise in the system or are part of the neural code itself remains to be determined.

484 SYNAPSES OF CRAB STOMACH MUSCLES: PHYSIOLOGY AND ULTRASTRUCTURE. C.K. Govind, H.L. Atwood* and S.S. Jahromi. (SPON: D.E. Meiss) Scarborough College, Dept. Zool., Univ. of Toronto, West Hill, Ontario, Canada M1C 1A4.

Intracellular recording from stomach muscles (GM8b, GM9) of blue crabs (Callinectes) reveals large EPSPs (5-20mV) in response to single impulses in the one axon innervating these muscles. Following a single impulse the EPSPs show a large facilitation which is long-lasting (10sec). Extracellular recordings at individual foci show quantal contents of 2 or more for events recorded during low frequency stimulation (0.5Hz or less). Compared with many other crustacean neuromuscular synapses, these show relatively high quantal content of transmission at low frequences of stimulation and long-lasting facilitation. Also these synapses are glutamate sensitive unlike synapses of the pyloric dilator muscle which are cholinergic as in the spiny lobster (Marder, Nature, 251, 730, 1974).

Three-dimensional reconstructions of the synapse-bearing terminals show that synapses occur on enlarged bulb-like processes which arise from very slender axons (<0.5μm diameter). The synaptic vesicles are generally round in contrast to those seen in terminals of the pyloric dilator muscle which are less regular (cf. King, Neurosci. Abstr. 1, 564, 1975). Numerous dense-cored vesicles occur in both types of terminals. Synaptic vesicles cluster at dense bodies in the presynaptic membrane. About half the number of synapses sampled were 1-3μm^2 in contact area, with the others ranging in size from 0.1-1μm^2. By contrast crayfish opener muscle terminals with low quantal content have synapses which seldom exceed 1μm^2 in contact area (Jahromi & Atwood, J. Cell. Biol. 63, 599, 1974). We suggest that larger synaptic contact areas may provide for higher quantal content of transmission at low frequencies of activation, by allowing more calcium to enter the synapse during the nerve impulse and by providing a larger area for transmitter release. (Supported by NRC and MDA of Canada).

485 INTERGANGLIONIC INTEGRATION OF DIFFERENT BEHAVIORAL COMPONENTS OF A CENTRALLY COMMANDED BEHAVIOR. W. Hening*, T. Carew, and E. Kandel. Div. Neurobiol. & Behavior, Columbia Univ., P & S, N.Y., N.Y. 10032

Respiratory pumping in Aplysia involves a coordinated contraction of the siphon, gill, and parapodia. The siphon and gill components of this behavior are mediated by motor neurons in the abdominal ganglion (Kupfermann et al., 1974). We now describe presumptive motor neurons in the pedal ganglia which contribute to the parapodial component. We have also investigated how the different behavioral components of respiratory pumping are integrated to produce the complex behavior. The siphon and parapodial components were examined in intact animals using videotape. During respiratory pumping the siphon and parapodial components are synchronous and the two parapodia are symmetrically activated. Cutting the pleuro-abdominal connectives abolishes the parapodial component. In a semi-intact preparation we have identified several classes of presumptive motor neurons which are symmetrically located in the pedal ganglia and receive synchronous input during respiratory pumping. The central command to these cells is synchronous with the central command (Interneuron II) to the siphon and gill motor neurons and is mediated by axons in the pleuro-abdominal connectives, some of which cross the pedal commissure to synapse bilaterally on pedal follower cells. Thus the command for respiratory pumping is confined to the abdominal ganglion and its projection to the pedal ganglia involves connections between abdominal interneurons and pedal effector neurons. The same pedal neurons activated during respiratory pumping are activated during reflex withdrawal and walking. This preparation may therefore be used to examine how the same group of effector organs can be integrated in different ways for the generation of different complex behavioral acts.

486 PHYSIOLOGICAL AND ANATOMICAL PROPERTIES OF CRICKET AUDITORY INTERNEURONS. R. R. Hoy* and G. Casaday* (SPON: M. Constantine-Paton). Cornell Univ., Section of Neurobiology and Behavior, Ithaca, N.Y. 14853

Auditory interneurons of the Australian field cricket Teleogryllus oceanicus have been mapped in the prothoracic ganglion by physiological and anatomical techniques ($CoCl_2$ filled micropipettes) that enable unique identification of specific neurons. Two classes of physiological units have been identified. In one class spike discharge is suppressed for the duration of a 5 kHz sound pulse and it responds with a vigorous discharge upon termination of the sound. The other physiological type (identified with at least four anatomical types) can be broadly classified as non-habituating pulse markers; they increase their firing rate at the onset of a sound stimulus and usually fire throughout the stimulation period. They code for a wide range of sound durations and show little habituation even after many minutes of stimulation; they often faithfully preserve the temporal pattern of the species calling song.

Neuroanatomical characterization of physiologically identified interneurons was carried out by cobalt chloride staining followed by silver intensification by the Timm's technique. In favorable cases it is possible to selectively stain the neuron from which the extracellular recording had been made. The dendritic field of the sound-suppressed unit projects to the ipsilateral auditory neuropile, its cell body is in the contralateral half of the ganglion, and it sends its primary axon up the cervical connective, presumably to terminate in the brain. In another physiological type the neuron geometry is complex: it sends projections to both bilateral neuropiles and has no primary axon; it is thus a presumptive intraganglionic interneuron. The remaining anatomical types each have unique but highly complex branching patterns; all send primary axons anterior, to the cervical connective.

487 PROPRIOCEPTIVE REFLEXES IN THE BUCCAL MASS OF APLYSIA. Behrus Jahan-Parwar. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

In preparations consisting of several isolated buccal muscles connected to each other only via buccal ganglia, stretching of one muscle results not only in a neural response (recorded via an passant suction electrodes) in the nerve that innervates that muscle but also in responses in the cerebral-buccal connectives and most other buccal nerves. These nerve responses are frequently followed by responses (tension changes monitored by transducers) in the corresponding muscles. Bathing the preparation in high (4 x normal) Mg^{++} solutions blocks all these responses except the neural response in the nerve that innervates the stretched muscle. Most stretch sensitive units appear to adapt slowly to a moderate (1-4 g) constant stretch. Quickly adapting units are also recruited during higher (> 4 g) stretch levels. Intracellular records obtained in the buccal ganglion from a motoneuron to the stretched muscle revealed that the stretch response in this neuron was mediated synaptically and was not due to deformation of the motoneuron terminals during passive stretch. High Mg^{++} blockage of synaptic transmission in the buccal ganglion alone inhibited stretch induced input to the motoneuron, but not muscle contractions produced by action potentials driven by intraneuronal current injection. These results suggest that proprioceptive reflexes are involved in the buccal mass activity of Aplysia.

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488 WHITE NOISE ANALYSIS OF CURRENT VOLTAGE RELATIONS IN APLYSIA NEURONS. D. Junge, J.A. Boles*, S. Dominguez-Villalpando*, and G.P. Moore. Dept. of Oral Biology, Sch. of Dentistry, UCLA, Los Angeles, CA 90024, and Dept. of Biomedical Engr., Sch. of Engr., Univ. So. Calif., Los Angeles, CA 90007.

The subthreshold current-voltage relations of neurons in the visceral ganglion of Aplysia have been investigated previously with current ramps, steps, and sinusoids. We have studied these same relations using a transmembrane current stimulus driven by a Gaussian white noise source, and have recorded the resulting transmembrane potential. Experiments were conducted on the R2 and R15 cell at 15°C. Using standard crosscorrelation algorithms we estimated the first- and second-order Wiener kernels characterizing the input-output relationship. The first-order kernel successfully estimates the response of the system to a pulse of current, and begins to show evidence of a local response as the input noise amplitude is increased. The second-order kernel indicates clearly the rectification properties of the membrane. When experiments were repeated at low temperatures, changes in the kernel characteristics, which we tentatively attribute to reduction of active transport processes and membrane conductance, were observed. (Supported by El Cholo Foundation.)

489 SURVEY OF CENTRAL NEURONS IN PLEUROBRANCHAEA. Richard M. Lee, Reinhard A. Palovcik* and George H. Dersham. Edsel Ford Institute, Detroit, MI. 48202.

Cells of the cerebropleural ganglion (brain) of the marine mollusk, Pleurobranchaea, were studied using three methods: cobalt "back-injection" dyeing, electrophysiological mapping with isolated ganglia, and studies of behavioral correlates using whole body preparations. The primary emphasis in this work was the identification of cells related to the control of feeding behavior. In the electrophysiological experiments with isolated ganglia, the synaptic inputs and axon pathways of 720 dorsal and ventral cells were determined. All of the input and axonal information for each cell, together with size, location, spontaneous firing pattern and other details was formatted and recorded on magnetic tape for computer retrieval and analysis. It was found that the location of brain cell bodies associated with different axon pathways (the 23 major brain nerves were used) determined by the electrophysiological methods agreed well with the results of cell dyeing, except for a few cases of cells distal to the point of dye entry. Correlational analyses indicated that stimulation of the oral veil nerves, the small tentacle n. and the rhinophore n. produced the largest and longest lasting response in most cells (see Lee and Liegeois, J. Neurobiol., 1974, p. 545, for anatomy). Neurons showing the greatest response to such stimulation had axons primarily in the mouth n. and large cerebropedal connectives; and in various other nerves to a lesser extent. In many cases, several seconds of inhibition preceded a long lasting excitatory response. Cells of this type had axons predominantly in the mouth n. Analyses of cells with multiple axons suggested numerous relationships for further investigation.

490 CYCLIC NUCLEOTIDES MAY MEDIATE THE EFFECTS OF PEPTIDES ON MOLLUSCAN NEURON ELECTRICAL ACTIVITY. Irwin B. Levitan and Steven N. Treistman. Friedrich Miescher-Inst., P.O. Box 273, Basel, Switzerland.

Vertebrate and invertebrate peptide hormones alter bursting rhythm in neurons R15 in Aplysia and 11 in Otala (Ifshin et al, Nature (1975) 254:72-74). We have found that cyclic nucleotide derivatives induce a similar effect in R15 and neuron F-1 in Helix. The vertebrate peptide hormones oxytocin and vasopressin, at concentrations similar to those required to alter bursting rhythm, cause an accumulation of cAMP in Helix and Aplysia ganglia. There is also an increase in cGMP, which occurs slightly later than that in cAMP. Peptide-containing extracts of Helix and Aplysia nervous system also alter bursting rhythm in R15 and F-1. These same extracts cause the accumulation of cAMP and cGMP in Helix and Aplysia ganglia. The cyclic nucleotide response is not observed in the isolated cell bodies of Aplysia neurons R2 and R15, suggesting that it may be confined to the ganglionic neuropil. The factor(s) in the extracts responsible for the cyclic nucleotide increase is heat and acid stable, dialysable, elutes in the exclusion volume of a Sephadex G-10 column, and is destroyed by pronase. In all these respects it is identical to the factor(s), tentatively identified as a peptide, which alters bursting rhythm. The data are consistent with the possibility that cyclic nucleotides may mediate the effects of peptides on neuronal membrane properties.

491 RESPONSES OF SPINY LOBSTER STOMATOGASTRIC MUSCULATURE TO GLUTAMATE AND ACETYLCHOLINE. C. J. Lingle* (SPON: D.L. Barker), Dept. of Biology, UO, Eugene, Oregon 97403.

The stomatogastric ganglion of the spiny lobster, Panulirus interruptus, contains 24 identifiable motor neurones innervating the striated foregut musculature. Acetylcholine is the best transmitter candidate for 11 of these neurones (Marder, Nature(Lond.)251:730(1974), J.Physiol.(Lond.), in press). The action of other possible neuroactive compounds has been examined on the muscles of this system.

On the basis of contractile responses to bath application of ACh or glutamate, the muscles so far examined can be classified into three distinct groups. Class I contracts in response to ACh, but not to glutamate (muscle/neurone;cpv1,cpv2/PD;gm2/GM;gm3 / LPG). Class II contracts in response to glutamate, but not to ACh (c7/AM;pl,cpv5/LP;cv2/IC). Class III, thought to be singly innervated (Maynard and Dando, Phil.Trans.B 268:161(1974)), contracts in response to both glutamate and ACh (gm6b/LG;gm9/MG;gm4c/DG). The glutamate-evoked contractures in Class III are temperature dependent, with a maximum response at 12°C and no contracture occurring above 19°C. Threshold for glutamate action at 15°C in both Class II and III is $5x10^{-5}M$ for onset of contracture, and $10^{-5}M$ for enhancement of nerve-evoked contractions. Intracellular recording from fibers of gm6b, a Class III muscle, demonstrates that both glutamate and carbachol produce depolarizations and, at sufficient concentration, diminution of nerve-evoked EJP's in the same fibers. In addition, two monoamines effect changes in the ongoing activity of the neuromuscular junctions. When nerve-evoked contractions are elicited by trains of stimuli, both octopamine ($5x10^{-8}M$) and 5-HT ($5x10^{-9}M$) enhance the evoked contractions on muscles of all 3 types (cpv2,pl, gm6b). The effect persists for 20-40 minutes after washout. Saturation of the action induced by one amine does not block the action elicited by the other. (Supported by USPHS Grant NS-10616.)

492 FUNCTIONAL DIFFERENCES BETWEEN CENTRAL EFFERENT PATHWAYS MEDIATING GILL WITHDRAWAL HABITUATION IN APLYSIA. K. Lukowiak* (Spon: C.M. Beiswanger) McGill Univ., Montreal, P.Q.

There are functional differences in central efferent pathways that mediate gill withdrawal behaviour in Aplysia. These differences were shown by the following experiments. (1) Repeated depolarization of L_7 (1/30 sec) with the ctenidial (Ct) and branchial (Br) nerves intact resulted in habituation of the elicited gill response. If Br was severed habituation did not occur; severing Ct had little affect on habituation. (2) Repeated depolarization of LGEV (1/30 sec) with Ct and Br intact resulted in an increment of the gill response. Cutting the Ct abolished this facilitation. (3) The interposition of activity in L_7 or LGEV during habituation (to tactile stimulation of the gill or siphon) resulted in dishabituation of the reflex; but only when Ct was intact. (4) Tactile stimulation of the siphon with Br, Ct and the siphon nerve (Sn) intact resulted in habituation of the gill reflex. If Br was severed repeated tactile stimulation of the siphon did not now result in habituation. If Ct and Sn were then severed the reflex once again habituated with repeated stimulation (mediated by the PNS). (5) When the siphon was surgically isolated from the gill (to remove PNS pathway) with Br, Ct and Sn intact repeated tactile stimulation of the siphon resulted in habituation of the reflex. If Br was then severed the reflex habituated at a slower rate, or didn't habituate at all. The results show that the peripheral terminations of the central efferents are sites of adaptive change and whose activity significantly contribute to habituation of the reflex. These terminations are functionally different, one set mediating decrement the other increment. Habituation may in part be the result of an interaction between these two processes.

MRC MA-5743.

493 ADAPTATION IN THE VENTRAL PHOTORECEPTOR OF LIMULUS: EVIDENCE SUGGESTING NON-PARALLEL EFFECTS ON RESPONSE AMPLITUDE AND LATENCY. John M. Martinez II* and Richard Srebro. Neurosensory Laboratory, Sch. Med. SUNY, Buffalo, N. Y. 14214.

Prevailing theory regarding mechanisms of adaptation in individual photoreceptors suggests a common basis for changes in the latency of the photoreceptor's response to light and its sensitivity (the amplitude of the response to a fixed intensity flash). It has been suggested that the concentration of intracellular calcium controls both of these response parameters. Intracellular recordings from the ventral photoreceptor of Limulus, however, indicate an apparent dissociation of light-dependent reduction in response amplitude and changes in response latency. Whether corresponding adaptive effects on latency and amplitude are observed appears to depend primarily upon the intensity of the flash. At moderate intensities, brief, repetitive light flashes result in a progressive reduction in response amplitude that is not accompanied by a progressive reduction in response latency. At considerably higher light levels, however, these parameters of the light response change together as predicted by theory. These data indicate that reductions in response amplitude caused by repetitive light stimulation with fixed intensity flashes may, or may not be accompanied by a progressive shortening of the photoreceptor's response latency. In this photoreceptor, therefore, adaptation may be shown to result in changes in both the amplitude and the latency of the response. However, it is unlikely that these response parameters simply represent different aspects of the same process.

494 THE EFFECT OF SENSORY DEPRIVATION ON THE DEVELOPMENT OF SYNAPTIC CONNECTIONS IN THE CRICKET CENTRAL NERVOUS SYSTEM. S. G. Matsumoto* and R. K. Murphey. Center for Neuroscience, SUNYA, Albany, NY 12222.

The response properties of a large primary sensory interneuron, the medial giant interneuron (MGI) of Acheta domesticus were examined after prolonged periods of deprivation. Specimens were unilaterally deprived of normal sensory input for the first seven (of 10) instars of post-embryonic development. The sensory elements are mechanoreceptive hairs on the cerci which respond to airborne vibrations. Chronic sensory deprivation was accomplished by immobilizing the mechanoreceptive hairs on one cercus with a thin film of facial cream (Clinique Cleansing Cream) following each molt. This procedure effectively blocks the major excitatory inputs to a single MGI.

A survey of the number of receptive hairs and cercal axons between the treated and control sides showed no significant difference. Recordings from cercal afferents and recordings from the entire cercal nerve revealed no differences in response of the receptors to tone pulses. Thus the periphery appears normal after this prolonged treatment. In contrast, the lack of a functioning sensory periphery results in a dramatic alteration of the input-output properties of MGI ipsilateral to the treated cercus. The deprived interneuron shows a drastic reduction in its excitatory response to ipsilateral cercal stimulation. In addition, an increase in an inhibitory input from its contralateral cercus was also found. We conclude that disuse of the cercal afferent to giant fiber pathway results in the modification of the synaptic efficacies of the afferent inputs. (Supported by NSF Research Grant #BNS 75-23454)

495 ANATOMICAL AND FUNCTIONAL SEGREGATION IN CRAYFISH OCULOMOTOR SYSTEM. Def. Mellon, Jr. and E.D. Lorton*. Dept. of Biology, Univ. Virginia, Charlottesville, VA. 22903.

Crayfish eye movements are controlled by at least four motoneuron groups in the brain. Three groups occur in the supraoesophageal ganglion: the giant cell cluster (GC); the lateral cluster (LC); and the anterior motor cluster (AMC). GC drives rapid eye withdrawal, and its axons exit via the optic tract. LC controls tracking eye movements, responding to rotation of either the visual environment or of the substrate beneath the walking legs. LC axons reach eye muscles via a separate, oculomotor nerve. AMC cells stabilize the eyes in the horizontal plane, responding in the dark to whole body roll or to roll of the substrate alone. AMC axons reach the eye via the optic tract in concert with GC axons. Behavioral studies by Fay (J. Comp. Physiol. Psych., 84:526, 1973) show that horizontal eye stabilization is also reflexively driven by unbalanced overhead illumination. AMC neurons may partially control this response. In addition, two motoneurons with cell bodies in the medulla terminalis supply a major dorsal suspensory muscle of the eyecup, muscle 12. These cells have different levels of tonic impulse activity in the dark; at least one is inhibited by light via the ipsilateral retina. These cells are probably responsible for light-induced horizontal eye stabilization. The crayfish oculomotor system thus exhibits anatomical segregation of neurons which is paralleled by separation of specific functional roles. Supported by USPHS grant NS 04989.

496 BURST GENERATION BY ELECTROTONICALLY COUPLED NETWORK.
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Functional organization and mechanisms underlying burst generation by a network of electrotonically coupled neurons (cyberchrons) has been studied in the snail Helisoma. The network controls the feeding behavior of the snail via identified motoneurons and constitutes a central program. Quantitative data acquisition and the analysis techniques of modern control theory have been used to investigate underlying mechanisms and organization with the aid of computer simulation. Utilizing this approach, the following principles have been demonstrated: The network consists of neurons organized into units (neuron in series with gap junction) which are functionally connected in parallel. The "loading" of the network through the electrotonic junctions influences the integrative and spiking behavior of the individual network members. An important consequence of the loading is that the frequency response of the network members is significantly greater than the frequency response of similar, but isolated neurons. The temporal and spatial summation of electrotonic synaptic input between network members is maintained at its most effective value. The increase in frequence response is essential to obtain sufficient regenerative excitation between network members to maintain the burst. The increase in frequency response is a unique property of electrotonically coupled networks and is not found in networks coupled with chemical synapses. It is believed that the frequency response increase concept is important to understanding why electrotonically coupled networks are found throughout the animal kingdom in situations requiring neurons to fire high frequency bursts of action potentials, such as required in escape responses.

497 NEURAL CONTROL OF SALIVARY GLANDS IN THE SNAIL, HELISOMA TRIVOLVIS, AND COORDINATION OF THEIR ACTIVITY WITH THE FEEDING CYCLE.
A. D. Murphy* and S. B. Kater. Dept. of Zool., Univ. of Iowa, Iowa City, IA. 52242.

The feeding cycle of Helisoma trivolvis is characterized (Kater, Am. Zool. 14: 1017-1036, 1974) by rythmic buccal mass contractions which are elicited by cyclical bursts of action potentials (APs) in motoneurons in the buccal ganglia. An identified pair of homologous neurons in the buccal ganglia (cells 4R & 4L) display rythmic bursts of APs coordinated with the activity of feeding motoneurons. These two cells are electrically coupled and each innervates the ipsilateral salivary gland. The paired salivary glands are basically tubular structures with the secretory cells arranged in discrete acini. When the nerve trunk which connects each buccal ganglion to the salivary gland is severed and the proximal stump back-filled with $CoCl_2$, the soma of cell 4 fills in every case. The distal stump of the nerve trunk may also be filled with cobalt to reveal the pattern of innervation of the gland. The actual glandular cells of Helisoma exhibit overshooting APs which may be greater than 100 mv. These APs in the salivary gland cells are elicited on a one to one basis by APs in cell 4. Hyperpolarization of these glandular cells may reveal underlying prepotentials that resemble classical EPSPs. High gain recordings from such cells may disclose spontaneous potentials that are essentially indistinguishable from miniature endplate potentials such as those seen at the neuromuscular junction. Only a small proportion of the glandular cells display such miniature potentials and it is only these cells which produce APs as a result of low levels of depolarizing current injection. This appears to represent a good example of 'impedance matching' in a biological system. Glandular cells are tightly electrically coupled so that APs, once generated, are propagated throughout the gland via low-resistance junctions.

498 NEURONAL CORRELATES OF SHORT-TERM HABITUATION AND SENSITIZATION OF THE SIPHON WITHDRAWAL RESPONSE IN FREELY-BEHAVING APLYSIA. H. M. Pinsker, J. S. Cobbs* and J. E. Kanz*, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

The siphon withdrawal response of Aplysia to repeated tactile stimulation undergoes centrally and peripherally mediated habituation and sensitization (Perlman, 1975). We chronically recorded multiunit activity underlying the central component of the siphon withdrawal response with extracellular cuff electrodes on the siphon nerve (Pinsker, Cobbs and Kanz, 1976). In order to correlate the behavioral response with the ongoing neuronal activity, we used a photocell and light source to monitor the amplitude of siphon withdrawal in unrestrained animals. Habitation and sensitization of the siphon response were seen before and after implanting the chronic electrodes. The directly triggered activity of some efferent units decreased during habituation and increased during sensitization. Direct stimulation of the siphon nerve provided a control for changes in neuromuscular efficacy in the intact animal.

A spontaneously occurring siphon contraction, attributed to a burst of activity in an unidentified cell, "Interneuron II", can also be activated by tactile stimuli (Perlman, 1975). In the chronic siphon record, this contraction is correlated with a stereotyped pattern of discharge involving the siphon motoneurons. A novel and intense stimulus can trigger a short-latency "Interneuron II response" that combines with the reflex response. With repetition, the latency of the Interneuron II burst can increase so that its associated contraction becomes a separate response. This process could provide another mechanism for habituation and sensitization of siphon withdrawal. Supported by NIH grants NS 11255 and NS 12223.

499 ELECTROPHYSIOLOGICAL CORRELATES OF LEG LIFT LEARNING IN THE COCKROACH. **Roger Lyons Reep and E.M. Eisenstein. Biophysics Department, Michigan State University, East Lansing, Michigan. 48824.**

Cockroaches can be trained to maintain a flexed leg position when leg extension is paired with leg shock. Yoked control animals do not show such learning. We have found that there are a few distinct types of behavioral responses to this training situation. Thus group averages of the data have been supplemented with an analysis of each animal's individual behavior.

The neuromuscular elements active in leg lift learning are part of the locomotory system of the cockroach and are amenable to electrophysiological investigation. Presently we are examining the ability of D_g , a slow excitatory coxal depressor motor neuron, to undergo changes in its spontaneous firing rate as a result of contingently applied shocks. The effect of randomly applied shocks is being determined as well.
This study is beginning to provide us with an understanding of the means by which different individual behavior patterns emerge in the behavioral leg lift experiments.

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500 REGENERATIVE ACTIVITY IN THE PRESYNAPTIC TERMINAL REGION OF THE BARNACLE PHOTORECEPTOR. W.N. Ross* and A.E. Stuart. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

We have studied with intracellular recording the membrane properties of the terminal region of the median photoreceptors of the giant barnacle, Balanus nubilus. These four large receptors respond to light with an initial depolarizing transient followed by a steady plateau. Following illumination, the membrane hyperpolarizes beyond the dark resting potential and slowly returns to rest. Both the depolarizing and hyperpolarizing responses are conducted electrotonically with high fidelity from the somata of the receptors, located in a peripheral ocellus, to their terminals in the supraesophageal ganglion often 10 mm or more away (Hudspeth, Poo, & Stuart, in preparation).

In the presynaptic terminal region the transient part of the receptor potential causes a small regenerative depolarization which can, on occasion, appear as a larger action potential of variable amplitude. 10^{-6} M tetrodotoxin has no effect on this terminal action potential, or on the receptor potential, although it blocks impulses in ganglion cells. 30 mM Co^{++} blocks the action potential reversibly. When the light response is below threshold for the regenerative event, the action potential can be elicited by bathing the terminal region in 15 mM 4-aminopyridine, 10 mM procaine, or 20 mM Ba^{++} instead of Ca^{++}. These pharmacological effects are consistent with a Ca^{++}-dependent regenerative mechanism similar to that described in muscle fibers of this species (S. Hagiwara, Advan. in Biophys. 4, 71, 1973). Simultaneous recordings from one receptor axon with two separated microelectrodes indicate that the action potential is generated locally in the terminal region and conducted backwards electrotonically. This regenerative event may play a role in synaptic transmission, or may be a manifestation of a mechanism which amplifies the light response. Supported in part by U.S.P.H.S. grant EY01188.

501 MAKING THE STOMATOGASTRIC GANGLION WORK. David F. Russell. Biology Dept., UCSD, La Jolla, CA. 92093.

The stomatogastric ganglion (STG) of the lobster contains pattern generators for the gastric and pyloric rhythms of the stomach. When isolated by itself, the STG rarely (<5% of all cases) produces the gastric rhythm, and then only intermittently; it produces a weak pyloric rhythm (1-2 sec period) while many neurons of the pyloric system do not fire. A way was sought of obtaining more reliable motor rhythms from the STG. As a general approach, in vitro preparations were made in which the STG was left attached to increasingly larger chains of more central ganglia. In this way, the commissural ganglia were identified as major sources of STG inputs. In "combined" preparations including the two commissural ganglia, the esophageal ganglion and the STG, the gastric rhythm has operated continuously for at least 4 hrs in 67% of all cases. The pyloric rhythm now typically has a 0.5 sec period and almost all neurons of the pyloric system fire spikes. To demonstrate this activation, spike conduction along the single unique input nerve to the STG (the stomatogastric nerve) was reversibly blocked by perfusing isotonic sucrose solution through a pool around the nerve. With inputs blocked, the gastric oscillator stopped and the pyloric rhythm slowed, but both were revived upon reversal of nerve blockade. To conclude, while the STG contains basic circuitry of the gastric and pyloric oscillators, connections with the commissural ganglia serve to make STG oscillators work.

(Supported by NIH grants to Dr. A. I. Selverston).

502 LOCALIZATION OF AND SPECIFIC RECEPTORS FOR PHENYLETHANOLAMINE IN APLYSIA. J.M. Saavedra, J. Ribas, J. Swann and D. Carpenter, NIMH and AFRRI, Bethesda, Md. 20014

Phenylethanolamine was detected in the nervous system of Aplysia. Specific receptors for this amine are also present. Phenylethanolamine was assayed by an enzymatic-isotopic technique (Saavedra, J.M., Anal. Biochem. 59, 628, 1974). It was found in all ganglia with the greatest amount in the buccal (4.6±0.4 ng/mg protein) and the lowest in the pedal (1.4±0.1 ng/mg protein). Of all of the regions of the ganglia, the neurophile showed the greatest concentration, approximately 5X that of other regions. The amine was also present in both connective (4.6±0.9 ng/mg protein) and posterior parapodial (2.8±0.8 ng/mg protein) nerves. It could not be detected, however, in any of the major identified neurons.

Specific receptors for phenylethanolamine were found in the nervous system of Aplysia. No responses were obtained after iontophoretic application of structurally related putative neurotransmitters (phenylethylamine, dopamine, norepinephrine, octopamine and histamine) from 5-barrel electrodes. Such receptors are rare, and most frequently found in buccal or cerebral ganglia. Three types of responses were found, due to Na+, Cl- or K+ conductance increases, respectively. The Cl-conductance response was most frequent. These results, demonstrating presence of and specific receptors for phenylethanolamine suggest that this amine may be considered to be a putative neurotransmitter in the nervous system of Aplysia.

503 ACTIVE HYPERPOLARIZING POTENTIAL IN PARAMECIUM AURELIA. Youko Satow. Lab. of Molecular Biology, Univ. of Wisconsin, Madison, WI. 53706.

Normal P. aurelia generates active hyperpolarizing potentials in a Ca-solution ($Ca(OH)_2$ 1 mM, citric acid 0.6 mM, pH 6.8-7.0) when stimulated with inward currents above 5×10^{-10} A. Bathed in this solution, the membrane rests at -30 mV. The active hyperpolarization brings the potential down to a trough of -120 mV. The rate of fall of this potential is small compared to the rate of rise of the well-known Ca-action potential. Externally applied TEA^+ (0.4 mM) or K^+ (0.4 mM) or internally applied TEA^+ through 2 min., 10^{-9} A outward current inhibits this active hyperpolarizing potential. Internally applied K^+ through 5 min., 10^{-9}A outward current enhances it. These results suggest that this active hyperpolarizing potential is due to the activation of a K channel and the action current is carried by K^+. The mechanism of depolarization return to rest and the possible involvement of anion will be discussed. P. caudatum shows similar active hyperpolarizing potential. Intracellular recording is made with 500 mM KCl filled, 130 MΩ electrodes. Supported by NSF BMS 75-10433 and PHS GM 22714-01 to C. Kung.

504 A MECHANISM FOR PRESYNAPTIC INHIBITION IN TRITONIA DIOMEDIA. M.T. Slawsky* and P.A. Getting* (SPON: J.G. Nicholls).
Dept. of Biol. Sci., Stanford University, Stanford, CA. 94305.

Presynaptic inhibition of monosynaptic chemical excitatory connections occurs in a primary afferent cell population of the mollusc, Tritonia diomedia. Hyperpolarizing synaptic potentials (primary afferent hyperpolarization or PAH), which result from chloride conductance changes in the afferent cells, mediate the observed presynaptic inhibition. When chloride ion substitution reverses the PAH to produce a primary afferent depolarization (PAD), there is little change in the efficacy of the inhibition. The conductance change associated with PAH shunts the afferent spike amplitude, and thus provides the major contribution to inhibition in this system. The observed spike shunting is similar to that caused by PAD during presynaptic inhibition in crayfish tactile afferents. This similarity between these two preparations suggests that the conductance change rather than the amplitude or the polarity of the associated voltage changes underlies presynaptic inhibition.

505 AFFERENT PATHWAYS TO GIANT INTERNEURONS IN THE EARTHWORM. Philip H. Smith* and Jay E. Mittenthal. Dept. Biol. Sci., Purdue Univ., W. Laf., IN. 47907

Withdrawal behavior in earthworms is mediated by giant interneurons. The medial giant is activated by stimulation of anterior segments, while the paired lateral giant (LG) fibers respond to posterior stimulation. We are characterizing afferent pathways to the giant interneurons in Lumbricus terrestris at anterior, central and posterior levels in order to understand the neuronal organization underlying anteroposterior specialization of response. During tactile or electrical stimulation of the body wall we record post-synaptic potentials (psp's) intracellularly from a giant fiber while monitoring impulses in side nerves of the ganglion (SNI-SN3). Four classes of afferent fibers responding to mechanical stimuli are known (see GÜnther, Z. Morph. Tiere 70:141 (1970); Verh. Dtsch. Zool. Ges. 64:261 (1971)): touch receptors; pressure receptors; proprioceptors; and unidentified tactile afferents. These can be distinguished according to adequate stimulus, pattern of impulse discharge during response, axon diameter, and distribution of axons in side nerves. We have noted that touch and pressure receptors elicit depolarizing psp's in posterior LG's. The psp's in response to pressure receptors often initiate LG impulses; psp's due to touch receptors are smaller. In all three side nerves unidentified afferent fibers generate small depolarizing psp's or smoothly graded depolarization. (supported by N.I.H. grant NS-11383 to J.E.M.)

506 SEXUAL BEHAVIOR IN TRITONIA DIOMEDIA: SENSORY AND MOTOR FUNCTIONS OF NEURONS INNERVATING THE GENITALIA. Robert W. Snow* (Spon: A.O.D. Willows) Dept. Zool., Univ. Wn., Seattle, WA. 98195 and Friday Harbor Labs., Friday Harbor, WA. 98250.

The nudibranch Tritonia diomedia can be reliably induced to copulate in the laboratory. To locate neurons involved in this behavior the genital nerve (RPdN 4; Willows et. al., J. Neurobiol. 4:207, 1973) and its symmetrical partner LPdN 4 were back-filled with cobalt. The genital nerve innervates the genitalia and the right lateral body wall while LPdN 4 innervates only the left lateral body wall. Several groups of neurons were found that are unique to the genital nerve.

Extracellular stimulation of the pedal-pleural connective through which the axons of these and other neurons run is followed by penis eversion after a 1 to 2 sec. delay. Repeated eversions can be elicited every 5 to 15 mins.

Intracellular recordings in both whole animal and semi-intact preparations from two groups of neurons unique to the genital nerve lying on the ventral aspect of the cerebral-pleural ganglion show that these groups contain motor neurons causing movements of the genitalia and surrounding body wall. Other neurons in these groups have no obvious motor function when stimulated individually. Most neurons in these groups are either excited or inhibited by tactile stimulation of the genitalia, but none have been found that are sensitive to touch of other body regions. In contrast, about 20 neurons in the pleural and pedal ganglia have been found to be sensitive to touch of both the genital and other body regions.

Work is in progress to determine the role of these neurons in copulatory behavior.

507 SEQUENTIAL ACTIVITY AS A CONSEQUENCE OF SYNAPTIC CONTROL OF ELECTROTONIC COUPLING AMONG NEURONS. D. C. Spray, M. E. Spira* and M. V. L. Bennett. Dept. Neurosci., Einstein Coll. Med., N. Y.

Expansion (Exp) and circumferential (Cir) motoneurons in the buccal ganglia of Navanax control the movements of prey engulfment and swallowing. Electrotonic coupling within each group is widespread, allowing synchronous activation of all members of each separate pool that leads to powerful expansion or constriction of the entire pharynx. Uncoupling by inhibitory inputs to each group of motoneurons allows asynchronous firing and this may allow Cir motoneurons to mediate asynchronous constrictions and Exp motoneurons to mediate regional expansions. Activation of Exp motoneurons inhibits Cir motoneurons by means of an unidentified neuron or neuronal group; Cir motoneurons appear to fire in rebound from this inhibition. Cir motoneurons form excitatory connections, probably electrotonic on a group of neurons that we call "C" that are inhibitory to the Cir neurons. The C group may be identical to the neurons mediating inhibition from the Exp motoneurons. Under conditions of low C group activity, Cir motoneurons are well coupled. When the C group is active there is an effective reversal in sign of coupling between two Cir motoneurons: in these circumstances depolarization and firing of one Cir neuron hyperpolarizes other Cir cells and hyperpolarization of the first cell depolarizes and can fire other cells. The connections from Exp to Cir motoneurons result in out-of-phase firing between these two groups of motoneurons; connections within the Cir motoneuron pool allow out-of-phase firing within the group. These sequential outputs may be important in generating the movements for prey capture and swallowing.

508 DOPAMINE MIMICRY AND MODULATION OF L7 GILL MOVEMENTS IN APLYSIA.
John W. Swann*, C. Nelson Sinback* and David O Carpenter. Dept. of Neurobiology, Armed Forces Radiobiology Research Institute, Bethesda, MD. 20014.

Dopamine (DA) has been reported in high concentrations in Aplysia gill (Carpenter et al., Internat. J. Neurosciences 2: 49, 1971). Experiments were undertaken to investigate a physiological role for this putative neurotransmitter in the gill. In 10 isolated gill preparations the parieto-visceral ganglion (PVG) was removed and the gill was infused with DA. The PVG was removed to avoid centrally mediated responses to DA. In all preparations DA produced contractions of efferent vessel trunklets (the vessels between the distal ends of the pinnules and efferent vessel) and pinnule longitudinal muscles. In 8 isolated gill preparations the threshold concentration of DA was 10^{-7}-10^{-6} M. In the remaining preparations higher concentrations, up to 10^{-4} M, were required. In semi-intact preparations of gill plus PVG, increased spiking of L7 produced efferent vessel trunklet and pinnule longitudinal muscle contractions identical to those induced by DA. In a third experimental protocol, semi-intact preparations were used in which the PVG was isolated during infusion of the gill with DA. In 15 such preparations DA at 10^{-7}-10^{-6} M produced identical contractions as in the isolated gill preparations. In all preparations firing of L7 during infusion of dopamine at threshold concentrations or higher potentiated the responsiveness of the gill to L7, as compared to L7 gill contractions before and after DA infusion. These results show that DA can modulate the responsiveness of the gill to L7 and is consistent with a possible role for DA as the neurotransmitter for L7.

509 MONOSYNAPTIC INHIBITION BETWEEN FLIGHT MOTONEURONS IN DROSOPHILA MELANOGASTER.
Mark A. Tanouye* (SPON: R.J. Wyman). Biol. Dept., Yale Univ., New Haven, Conn. 06520.

During tethered flight, the motoneurons innervating the indirect flight muscles of Drosophila are active in characteristic output patterns. Harcombe and Wyman (in preparation) used antidromic stimulation techniques to demonstrate inhibition between flight motoneurons. The motoneurons innervating different fibers within a single muscle inhibit each other. The characteristics of this inhibition were shown to be essential in creating the observed flight motor pattern.

In the present study, the activity of flight motoneurons was recorded during normal tethered flight. Inhibition occurs with a latency of .6 msec., indicating a monosynaptic inhibitory pathway.

To determine if this inhibition was driven via motoneuron collaterals, individual flight motoneurons were stimulated antidromically by passing current in identified muscle fibers. The antidromic activation of the motoneuron was verified in a dissected preparation by en passant recording from the wing nerve while stimulating a single muscle fiber. The stimulating electrode simultaneously recorded the muscle fiber spike. The muscle spike and motoneuron spike were initiated at the same time. Single motoneurons were then stimulated during flight. Inhibition of motoneurons innervating other fibers within the same muscle occurs within .6 msec.

These experiments indicate that inhibition between flight motoneurons occurs with a monosynaptic time course and is mediated by motoneuron collaterals.

510 THREE PHARMACOLOGICALLY DIFFERENT POTASSIUM CURRENTS IN MOLLUSCAN CELL BODIES. Stuart H. Thompson*(SPON: B. Sakitt). Dept. Zoo. and PBio., Univ. Washington, Seattle, WA., 98105.

Voltage clamp studies of molluscan nerve cell bodies have revealed complicated outward current waveforms. Pharmacological experiments indicate that the outward currents seen during depolarizing voltage clamp steps in Tritonia cells represent three distinct and separable potassium channels. A transient outward current, named A-current by Connor and Stevens (J. Physiol. 213:21,1971), is blocked by externally applied 4-aminopyridine (4-AP). 50% reduction in A-current amplitude occurs at a 4-AP concentration of 1.5mM. 4-AP does not effect delayed outward currents. Tetraethylammonium ion (TEA) also reduces A-currents, causing 50% block at a concentration of 100mM. TEA is much more effective in blocking the voltage-dependent component of delayed outward current; producing 50% block at a concentration of 8mM. A third component of potassium current, called C-current, is insensitive to externally applied TEA or 4-AP but is blocked by the Ca++ antagonists Co++ and Mn++ and by Ca++ free saline. C-current is similar to the Ca++ dependent potassium current described by Meech and Standen (J. Physiol. 249:211,1975). These cells therefore have three different potassium conductance systems. Multiple outward current channels confer some interesting special properties to the cells such as the ability to fire repetitively at low frequency, to adapt to constant stimuli, and to show a pronounced post-stimulus hyperpolarization.

511 LONG-TERM SYNAPTIC INHIBITION IN A MOLLUSCAN NEURON IS AFFECTED BY TREATMENTS WHICH ALTER CYCLIC NUCLEOTIDE LEVELS. Steven N. Treistman and Irwin B. Levitan. Friedrich Miescher-Inst., P.O. Box 273, Basel, Switzerland.

The endogenous bursting rhythm of neuron R15 in Aplysia abdominal ganglion is subject to long-term synaptic inhibition following very few presynaptic stimuli. We have examined the possible involvement of cyclic nucleotides in the hyperpolarization produced in R15 by stimulation of the branchial nerve. Stimulus intensity was adjusted so that single stimuli produced a biphasic post-synaptic potential, with the predominating hyperpolarizing phase lasting 30-90 seconds. Perfusion of the ganglion with the phosphodiesterase inhibitor, theophylline (1 mM) caused an augmentation of both the depth and duration of the hyperpolarizing phase. Within an hour of washing, the synaptic potential had returned to control values. Although a presynaptic action of the drug cannot be ruled out, postsynaptic potentials were unaffected in two other neurons which respond to branchial nerve stimulation. Injection of guanylylimidodiphosphate, an activator of adenyl cyclase, into R15 resulted in a non-reversible hyperpolarization within 2 minutes. We have determined that guanylylimidodiphosphate does stimulate adenyl cyclase activity in homogenates of Aplysia abdominal ganglion. The data are consistent with the notion that synaptically-induced long-term inhibition in R15 may be mediated by cyclic AMP.

512 INTRACELLULAR STUDIES OF CRAYFISH CLAW MOTONEURONS. T.J. Wiens* and H.L. Atwood. Dept. Zool., Univ. of Toronto, Toronto, Ont. , Canada M5S 1A1

An isolated preparation of the chelipeds and first thoracic ganglion of the crayfish retains reflex activity and permits intracellular recording and cobalt injection. Recordings from the somata of the claw motoneurons show decremented axon spikes of 5-10 mV and subthreshold synaptic activity of similar amplitude. Current (~20 nA) injected into the soma elicits repetitive firing (10-20/s) in all motoneurons except the fast closer excitor (FCE). Synaptic potentials recorded in these cells are consistent with connections inferred from spike train analysis (Wiens and Gerstein, J. Neurophysiol. 38, 909, 1975). Thus, spikes in the opener inhibitor (OI) are accompanied by IPSP's in the opener excitor (OE) and by EPSP's or spikes in the slow closer excitor (SCE). SCE spikes are accompanied by spikes or EPSP's in OI. Further, FCE receives sensory input parallel to that of SCE and OI during claw reflexes and may often fire dendritic spikes in response, although axonal spikes are not easily elicited.

Cobalt injection reveals a structural organization parallel to the functional one. The major dendrites of the three synergistic closing effectors (FCE, SCE, OI) are given off closely together and branch in register;they encircle the base of the larger (posterior)nerve root to the limb, which contains their axons as well as those of the tactile and proprioceptive afferents that elicit closing reflexes. OE's structure is quite different, with dendrites confined largely to the dorsal surface of the ganglion.All four motoneurons lie totally within the ipsilateral hemiganglion, in keeping with their unilateral reflex behavior. Their somata lie on the edge of the connective,OI's at the posterior and the excitors' at the anterior end of the ganglion. The size ranking: OI<OE≤SCE=FCE holds for the injected and fixed somata. Thus, the "size principle", relating increasing motoneuron phasicity with increasing soma size, does not appear to apply to the two closer excitors.

513 EFFECTS OF PICROTOXIN AND CURARE ON CENTRAL INHIBITION IN THE CRAYFISH. Lon A. Wilkens and John A. Baker*, Dept. of Biology, University of Missouri-St. Louis, St. Louis, MO 63121

The crayfish caudal photoreceptor (CPR), in addition to its anomolous light sensitivity, is a higher-order somatosensory interneuron (D. Kennedy, JGP 46:551, 1963). Recent evidence indicates that peripherally-induced inhibition of spontaneous CPR activity is mediated indirectly by way of other 1st-order central interneurons (G. Marzelli and L. Wilkens, Amer. Zool. 16:179, 1976). Evidence is presented here which suggests that central inhibition is due to the release of γ-amino butyric acid (GABA) as a chemical neurotransmitter. In the isolated and continuously perfused 6th abdominal ganglion of the crayfish, Procambarus clarkii, inhibition was produced by stimulating axons in the central connectives and in the peripheral roots. Both sources of inhibition could be blocked by perfusion with picrotoxin. Threshold for complete loss of inhibition was seen at picrotoxin levels as low as 3×10^{-6} molar. Perfusion with 3×10^{-5} molar d-tubocurarine also blocked inhibition via the peripheral roots but did not block inhibition effected centrally. These data support the evidence for polysynaptic central inhibitory pathways in the crayfish and suggest that GABA is an inhibitory transmitter centrally as well as peripherally. Our evidence also supports the functional role of acetylcholine as a sensory neurotransmitter. Supported by NIH grant NS12971-01.

514 CAN FUNCTIONAL SHIFTS OCCUR DURING EVOLUTION OF SERIALLY HOMOLOGOUS MOTORNEURONS? John A. Wilson* (SPON. A. Strickholm). Dept. of Biology, Univ. of Oregon, Eugene, OR. 97403

Serially homologous motorneurons innervating muscles serving different functions occur in arthropods, such as the locust, Schistocerca gregaria. The structures and functions of metathoracic leg motorneurons are known. Those of the pro- and mesothoracic ganglia were examined functionally and also anatomically by intracellular staining. They were then compared with their metathoracic equivalents.

The major change which is seen in these cells occurs in the structure of the fast and slow extensor tibiae (FETi and SETi). Pro and meso FETi and meta SETi have cell bodies located near the point of entry of the anterior connectives, have similarly shaped neurites which give off branches in similar locations, and leave the ganglion by nerve 3. Pro and meso SETi and meta FETi have cell bodies located in a more posterior position, have a loop in their primary neurite from which major branching towards the center of the ganglion originates, and leave the ganglion by nerve 5. It follows that SETi and FETi have switched either their location or their functional roles. It is considered that the latter is more probable. Hence it is important to have functional as well as structural data when discussing relationships between anatomically similar neurons. (Supported by PHS grant numbers 5-T1-GM-00336 and 1-T23-GM07257)

515 RELATIONSHIP BETWEEN NEGATIVE RESISTANCE REGION AND SLOW OUTWARD CURRENT IN APLYSIA BURSTING NEURONS. W.A. Wilson, D. Johnston, and M.T. Clark, Epilepsy Ctr. VA Hosp. Durham, NC, 27705 and Neurol. Dept. U. of Mn., Mpls., Mn. 55455

Molluscan bursting neurons have drawn increasing attention in recent years as a possible model for the neuropharmacological activity of different chemical agents. Considerable uncertainty exists, however, concerning the mechanisms responsible for bursting or slow wave activity. This study was specifically directed towards elucidating the relationship between the inward current and the slow outward current which depolarizes and hyperpolarizes the cell respectively. Identified bursting neurons from Aplysia (L3, L6 and R15) were voltaged clamped with either double or single microelectrode clamping systems. The complete current-voltage (I-V) curve of the cell was obtained by using 5 or 10 second step commands. The data reveal three distinct regions in the I-V curve: a) a linear portion from approximately -50 mV to more hyperpolarized potentials, b) a negative resistance region from approximately -50 mV to -30 mV, and c) a linear region from about -30 mV to more depolarized values. The first linear region from -50 mV is a potassium conductance which is insensitive to temperature, TEA, and calcium blocking agents. By applying a 5 or 10 second depolarizing conditioning pulse before each test command the negative resistance region in the I-V curve is eliminated. The negative resistance region and the slow outward current are not separable mechanisms. Each shows sensitivity to external potassium, calcium blocking agents, DNP, temperature and insensitivity to TEA. These and other data force us to conclude that the events responsible for the negative resistance and the subsequent activation of the slow outward current are due to a common origin. (Supported by VA 9459, Durham NC, Mn. Med. Fd. and Grad. Sch. U. of Mn.)

516 NEURONS INNERVATING SEX ORGANS IN THE LEECH. Birgit Zipser. Dept. of Physiology, State University of New York, Downstate Medical Center, Brooklyn, N.Y. II203.

The neural network underlying the operation of sexual organs is being studied in the leech. Two of the 34 ganglia of the leech nervous system innervate the genitalia. One of the two, the caudal sex ganglion, sends two special nerves to the male sexual organs. It contains extra neurons not present in the standard midbody ganglia. Seven new neurons have been identified so far, all of which are activated by the two nerves that also provide input to standard neurons like the Retzius cells.

A pair of two homologous neurons that elicit penile motion have been studied in detail. The two neurons are present in three species of leeches that differ in the complexity of their sexual apparatus. Each neuron sends its axon out through a different one of the two sex nerves. They receive synchronous inputs, afferent inhibitory back from the same sex nerves and excitatory from the head connective.

The neurons are not electrotonically coupled in a significant fashion, but they can be linked by a long-latency postsynaptic excitation. This reciprocal excitation synchronizes them when they are both spontaneously active. If at least one neuron is silent, the connection is labile or even absent.

The only feature in which the pair of homologous neurons is found to differ so far in the three species has to do with their spike parameters. The detailed nature of this electrophysiological response is correlated with the specific morphology of the male sex organs.

Limbic System

517 ONTOGENY OF THE HIPPOCAMPAL REGION IN THE RAT. S. A. Bayer. Dept. Biol. Sci., Purdue Univ., W. Lafayette, IN. 47907.

Cytogenesis in the rat hippocampal region was analyzed autoradiographically after ^{3}H-thymidine injections during either the prenatal or postnatal periods. The percentage of cells labelled by each series of injections was determined at 60 days of age in anatomically matched sections. By means of progressively delayed comprehensive labelling, the times of formation for each cell population could be determined.

Proliferation in the hippocampal region is very active in all areas on gestation day 15. By gestation day 18, 95% of the cells in the entorhinal cortex have formed. 94% of the cells in the subiculum form by gestation day 19. Both CA1 and CA3 pyramidal cells complete their formation by gestation day 21. However, CA3 adds only 29% of its population between gestation days 19 and 21, while CA1 adds 50%. Although dentate granule cells can be labelled with prenatal injections, 85% can also be labelled with a series of injections starting a few hours after birth. Over 40% of the population forms during the first week, 26% during the second week.

The various times for cytogenesis in the hippocampal region can be used to interpret some morphogenetic events in its formation. A cortical plate appears in the presumptive entorhinal area by gestation day 16. There is no cortical plate in the presumptive Ammonic area, but rather, a thin layer of pyramidal cells suddenly appears between gestation days 20 and 21. The dentate primordium makes its appearance as early as gestation days 16-17, when a stream of migratory cells can be seen leaving the neuroepithelium near the outgrowth of the choroid plexus. These cells accumulate (and proliferate) into a ball-like structure within the concave portion of Ammon's horn. The dentate granular layer begins on gestation day 20 as a thin layer of cells (the ectal arm) opposite CA1. By birth, the layer has lengthened to form a crest, then curves around the CA4 tip of Ammon's horn to form the endal arm. The first 2-3 weeks postnatally, the granular layer increases in depth.

During the adult period, the hippocampus continues to grow in size mainly by increasing its length in the septotemporal direction. Packing density of the pyramidal cells decreases along this plane, while that of the granule cells remains constant by continuing formation of granule cells in the adult.

518 INTERACTION BETWEEN NEONATAL SEPTAL LESIONS AND SUBSEQUENT MALNUTRITION: BEHAVIORAL EFFECTS. Wail A. Bengelloun, Peter J. Donovick and Richard G. Burright*. Dept. Psychol., SUNY, Binghamton, N.Y. 13901.

The effects of septal lesions and malnutrition on behavior show an important degree of similarity, particularly where activity, passive avoidance acquisition and reactivity to stimulation are the dependent variables. This study was designed to assess the influence of the interaction of these two manipulations early in life on subsequent behavior.

Half of the offspring of each sex in each of 28 litters of albino rats received lesions restricted to the septum at 24 hrs. of age. The other half underwent only the hypothermia (anesthesia) phase of the surgical procedure. Following surgery, pups were returned to their mother and left undisturbed until 10 days of age, when maternal retrieval tests were conducted and showed no differential preference for control or operated pups. At weaning (25 days of age) no significant differences among groups were found either in terms of activity or the number of transitions between levels of a 2-tier open field.

Immediately following open field testing, rats were assigned to housing and nutritional conditions such that a 2x2x2x2 completely randomized factorial design was attained. Four subjects of the same sex were housed in each cage, such that all rats in the cage had the same neonatal surgical treatment (i.e., 4 septals or 4 controls) or mixed neonatal surgical treatment (2 septals and 2 controls in the same cage). Half the subjects in each of these conditions received a commercially prepared diet with 8% protein while the other half received a comparable diet with 25% protein. These dietary conditions were maintained for 5 weeks, at which time half of each of the 16 groups were sacrificed for organ weights and histological purposes. The other half of the animals were housed singly and allowed 7 weeks of undisturbed recovery on a diet of Purina laboratory rat chow.

Those rats maintained on 8% protein and sacrificed at 60 days of age exhibited smaller absolute wet weights of whole brain, adrenal glands, liver, spleen and gonads. However, 8% protein animals had heavier brains and adrenals relative to their body weight. The higher adrenal weight/body weight ratio was more pronounced in males, particularly those without neonatal septal lesions.

Subsequent to the 7-week dietary recovery period on Purina chow rats remaining in the experiment were tested in a battery of behavioral tasks. In the open field, whereas female septals were more active than female controls, there was no lesion effect in males. Malnutrition did not appreciably alter activity, but mixed housing generally resulted in increased activity across treatments. In response to a flashing light, male activity was suppressed to a greater extent than that of females. Across sex, malnourished septal rats suppressed their activity less if they had been housed under the mixed condition. In a passive avoidance task utilizing shock as the aversive stimulus, no differences between groups were observed. These data point to an important degree of interaction between sex, neonatal septal lesions, malnutrition and housing conditions in determining subsequent behavior. Furthermore, the nature of these interactions appear to be task dependent.

519 DIFFERENTIAL ONSETS OF RAT SELF-STIMULATION BEHAVIOR ACROSS LOCI:MONOPHASIC PULSE PAIR ANALYSIS. R.J. Bodnar*, S.J. Ellman, S.S. Steiner*, P.A. Ippolito*, J.M. Healey*, G. Rodriguez*, W.T. Nelson*, M. Brutus* and E.E. Coons*. (SPON: D.D. Kelly). Psych. Depts., CUNY and NYU, New York, New York 10031.

Intracranial self-stimulation (ICSS) behavior can be parametrically manipulated by employing the monophasic C-T pulse pair stimulation technique which delivers trains of pulse pairs, the initial pulses of each pair designated as C pulses and the second pulses of each pair designated as T pulses. This technique allows systematic manipulation of the temporal distribution of succeeding C pulses (C-C interval), the temporal interval between C and T pulses (C-T interval), pulse durations, train duration, current amplitude and whether or not the T pulses are omitted. Several ICSS studies demonstrated that medial forebrain bundle ICSS response rates elicited at short (0.3-1.2 msec) C-T intervals are similar to rates elicited when the T pulses were omitted and significantly lower than rates elicited at longer (1.2-10.0 msec) C-T intervals. The present study investigated the relationship between C-T intervals and ICSS response rates across sites in stereotaxically-implanted animals with bipolar electrodes aimed at the locus coeruleus, substantia nigra, mid-ventral periaqueductal gray, medial forebrain bundle/perifornical area, hypothalamic internal capsule/fields of Forel and septum. Each ICSS site was tested at a 30 msec C-C interval and a 700 msec train duration. A current amplitude was used which elicited high ICSS response rates at a 5.0 msec C-T interval and operant (less than 10) rates in the T pulses-omitted condition. Eight C-T intervals (0.5, 0.8, 1.0, 1.2, 1.5, 2.0, 3.0, 5.0 msec) and the T pulses-omitted condition were tested for ICSS daily over each of nine days in a Latin Square design. A line of best fit was determined for each site function by the method of averages and an overall best fit line was gained for all animals in each site. Septal and locus coeruleus placements demonstrated significantly longer onsets of ICSS behavior as measured by C-T interval as compared to all hypothalamic or mesencephalic tegmental sites. All ICSS response rate onsets were also affected by whether the anodal path of stimulation was through either a cortical screw indifferent or the other pole of the bipolar electrodes. These results are discussed in terms of neuroanatomical specificity of ICSS sites.

520 SPATIAL CHARACTERISTICS OF HIPPOCAMPAL UNIT ACTIVITY

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A recent theory of hippocampal function (O'Keefe,J. and Dostrovsky,J., Brain Research , 34, 1971, 171-175) has suggested that the best way to describe hippocampal unit activity is as a function of the animal's location or place. To assess these spatial characteristics, single-cell activity in the dorsal hippocampal formation of rats was examined during the performance of a spatial discrimination task. This task, sampling each arm of an eight-arm radial maze for food reinforcement, was designed explicitly to maximize the spatial aspects of animal's behavioral organization.

The neural and behavioral data were stored on magnetic videotape during performance of the task with: 1) normal maze position and ambient light level; 2) reduced ambient light; and 3) axial rotations of the maze relative to the recording chamber. The data were analyzed by experienced observers in a split-half reliability format and verified by a computer rate-location analysis.

Of the 30 cells examined, 28 markedly altered their rates of firing in relation to the animals' position in well-defined spatially restricted areas. Spatial fields, typically limited to areas within a single arm, were found for all arms of the maze and for the central platform at the junction of the arms. Variations in ambient light, the execution of specific motor responses (e.g. grooming, drinking, etc.) or application of simple stimuli (e.g. tactile, olfactory, auditory, etc.) were not sufficient to appreciably change cell activity.

Axial rotation of the maze revealed three populations of spatial cells. Intramaze cells responded to specific areas of the maze regardless of the position of the maze within the recording chamber. Extramaze cells responded to specific area of the room, regardless of the specific arm occupying that location. Conjunction cells typically had several spatial fields, each influenced by a combination of intramaze and non-intramaze factors. Extramaze cells (about 45%) were concentrated in regio superior; intramaze cells (about 45%) were primarily in regio inferior; conjunction cells (about 10%) were distributed throughout the hippocampal gyrus.

For all cells, the spatial characteristics were highly visible, reliable over repeated samples of the same maze space, stable over the series of manipulations, and perseverant over extended periods of time.

These data are seen as supporting the hypothesis that the hippocampus is intimately involved in spatially-organized behaviors.

521 EFFECTS OF RITALIN HCL ON RATS RECEIVING FOCAL HIPPOCAMPAL X-IRRADIATION. Gary Freeman*, Robert B. Wallace and Phil Smith*. Dept. of Psychology, University of Hartford, West Hartford, Ct. 06117.

Previous research dealing with focal neonatal X-irradiation of the rat hippocampus has shown that it is possible to destroy up to 80% of the post-natally forming granule cells of the dentate gyrus. Paralleling this reduction in granule cell numbers, a variety of behavioral changes such as increased activity and an inability to inhibit responding have been noted. Since some of the behavioral deficits noted have borne at least superficial similarity to portions of the hyperkinetic syndrome in children, an experiment was carried out to assess the effects of Ritalin HCL on behavior in this hippocampal presentation. Ritalin as a stimulant was selected due to its beneficial effects when prescribed for hyperkinetic children.

Nine experimental animals (male Long-Evans hooded rats - random bred in our laboratories) were exposed to focal hippocampal X-irradiation - 150 r per day from days 2-15 past partus; examination of anatomical control subjects indicated that this irradiation schedule resulted in approximately a 70 to 80% reduction in the population of granule cells in the dentate gyrus without directly affecting the other cellular components of this region. Nine additional males served as non-irradiated controls. Within each of the above conditions, five of the nine animals were assigned to the drug group and the remaining four to the placebo condition. Ritalin was administered at a dosage of 8 mg/kg. IP for two days prior to testing and then for the duration of the testing schedule each day. All animals were run in a modification of a Porteus maze; time taken to complete the maze, errors made and reversals were recorded for all animals. Results indicated that the hippocampal animals as a group made fewer errors than did the control subjects--however, no significant differences with regard to time were noted. Across the drug-placebo conditions, more reversals were seen in those animals receiving the Ritalin HCL. The superiority of the hippocampal animals in the task was interpreted in terms of a cueing model for hippocampal functioning. Rather than producing the paradoxical effect seen with hyperkinetic children, the drug appeared to produce an overall elevation in arousal in both groups with, however, the effects being less pronounced in the hippocampal animals. Possibly our failure to confirm the human experimental literature could be due to drug level/task interaction effects.

522 BLOCKADE OF HYPOTHALAMIC SELF-STIMULATION BY PICROTOXIN INJECTION IN THE VENTRAL TEGMENTUM. Ernest W. Kent and Paul D. Fedinets*. Dept. Psychol., Univ. of Illinois, Chicago Circle, Chicago, Ill. 60680.

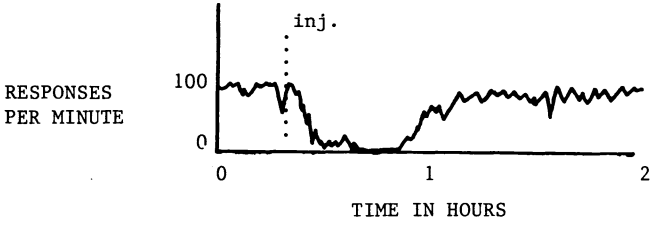
Whole body injections of picrotoxin or bicuculline in doses that do not disrupt lever pressing at comparable rates for escape from footshock has been reported to eliminate hypothalamic self-stimulation (Kent and Fedinets, Brain Research, in press.) To clarify the nature of this effect, intracerebral injections of picrotoxin and other compounds were administered in 0.5 ul of saline into the ventral tegmentum of rats self-stimulating through fine monopolar electrodes in the medial forebrain bundle and at the anterior tip of the locus coeruleus.

Doses of 50 nanograms of picrotoxin were sufficient to eliminate self-stimulation from these sites when injected unilateraly into the substantia nigra, ventral tegmental nucleus and area A10. Doses of 30 nanograms showed a clear depression of response rates. Injections placed 0.5 mm above these sites, or injections falling anterior to the ventral tegmentum produced no effect or markedly reduced effects. The doses which eliminate self-stimulation do not produce overt seizure or disrupt lever pressing for escape from footshock at 60 responses per minute.

Higher doses of picrotoxin produce rotation (contralateral in zona reticulata, ipsilateral in ventral tegmental nucleus), then stereotypic behaviors, and ultimately seizure. Injection ipsilateral to the stimulating electrode appears to be more effective than contralateral injection, but the difference is not marked. The animals are not sedated , and occasionaly return to the lever, but do not maintain responding."Priming" stimulation is activating during the drug effect, but not effective in shaping responding.

The action of picrotoxin is puzzling since the GABA agonist amino-oxyacetic acid (whole body, 25 mg/kg) or GABA injection into the ventral tegmentum do not facilitate self-stimulation and depress it at high doses However, the GABA agonist Lioresal mimics the effect of picrotoxin on self-stimulation when injected at the same sites in nanogram quantities (although other behavioral effects were not identical with picrotoxin). Neil (personal communication) has found that similar effects are obtained with nanogram quantities of strychnine, but that glycine is without effect.

TYPICAL RESPONSE TO IPSILATERAL INJECTION OF 50 NANOGRAMS OF PICROTOXIN.



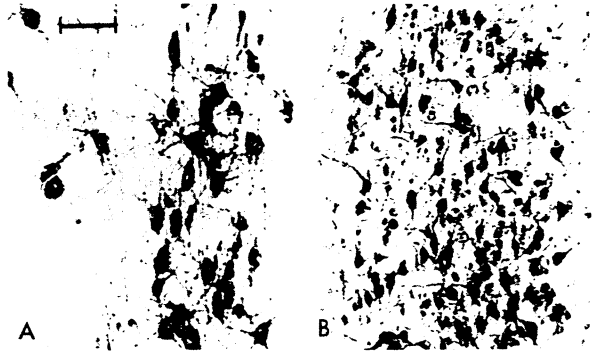
523 VENTRAL BUNDLE HINDBRAIN LESIONS ON LATERAL HYPOTHALAMIC AND LOCUS COERULEUS INTRACRANIAL SELF-STIMULATION. L. Mattiace*, A. Tempel*, J. Halperin*, P. Ippolito*. (SPON: S. J. Ellman). The City College of the City University of NY 10031.

Recent histofluorescent studies have shown that the ventral bundle, part of the ascending noradrenergic system, innervates the hypothalamus, including areas that support intracranial self-stimulation (ICSS). In order to determine the the contribution of the ventral bundle on lateral hypothalamus and locus coeruleus ICSS, some of the hindbrain areas containing the noradrenergic nuclei from which this bundle originates, ie. A7, A5, and the subcoeruleus, an area ventral to the principle locus coeruleus were lesioned. Twelve Ss were implanted ipsilaterally with bipolar electrodes aimed at the hypothalamus and one of the above mentioned sites. In a number of Ss, a third electrode was aimed at the locus coeruleus. After 8 days of baseline rate-intensity functions, Ss were then lesioned through the ventral hindbrain electrode with radio frequency current. Postlesion, Ss were tested daily for ICSS in the hypothalamus and locus coeruleus for a maximum of 56 days.

Postlesion, differential effects upon hypothalamic ICSS was obtained in 10 of the 14 Ss. These effects appear to be independent of the level at which the ventral noradrenergic pathway was lesioned. In five Ss that demonstrated increases in hypothalamic ICSS rates, ICSS electrodes impinged on the anterior medial forebrain bundle (MFB). In five other Ss that showed a decrement in hypothalamic ICSS rates postlesion, ICSS electrodes were localized dorsal (zona incerta) or lateral (crus cerebri) to the MFB. In two Ss where there was no substantial changes in rate-intensity ICSS postlesion, electrodes were localized between the MFB and crus cerebri-internal capsule area. The extent of lesion damage seems to be uniform in the disruption of processes, histofluorescently designated to be the areas of A7, A5, and the subcoeruleus. In conclusion, the differential effects upon hypothalamic ICSS appear to be independent of the level at which the ventral noradrenergic pathway was lesioned, and determined by the specific hypothalamic area under stimulation.

524 ACETYLCHOLINESTERASE-CONTAINING NEURONS IN THE MEDIAL TELENCEPHALON AND THE DIENCEPHALON OF THE MONKEY (Macaca mulatta). A. Parent, R. Boucher* and L.J. Poirier. Neurobiology Lab. and Dept. Anat., Laval Univ. Quebec, QUE. Canada G1K 7P4.

A pharmaco-histochemical method that involves staining for acetylcholinesterase (AChE) (Karnovsky-Roots procedure) at various times after administration of di-isopropylfluorophosphate (DFP) (Butcher et al., J. Neural Transm. 37: 127, 1975) was used to study the distribution and morphological organization of AChE-containing neurons in the CNS of the rhesus monkey. The light microscope study of the medial telencephalon and diencephalon of four monkeys sacrificed 4, 10, 12 and 18 hours after DFP (0.2 - 0.4mg/kg) has revealed the following facts. At medial telencephalic level, large neurons that stain intensely for AChE were encountered in the bed nucleus of the anterior commissure, the nucleus of the diagonal band of Broca and the substantia innominata (nucleus basalis of Meynert). The cell groups of nucleus basalis and of the nucleus of the diagonal band of Broca which are continuous with one another at the base of the forebrain, are chiefly composed of large and multipolar neuronal somata that stain intensely for AChE. In contrast, most neurons of the septal nuclei and of the indusium griseum are unreactive or weakly stained. At the level of the thalamus, the strongest AChE staining was found within the perikarya of nucleus anterior dorsalis and in most nuclei located in the lamellar or fibrous thalamic structures i.e., nucleus reticularis, the intralaminar nuclei (centre-median/parafascicular, centralis medialis, centralis lateralis, paracentralis and limitans) and the midline nuclei (paraventricularis, verticalis and centralis densocellularis). In most of these nuclei the perikarya are of medium-size and stain moderately for AChE. Their proximal processes are either lightly stained or devoid of AChE. At the level of the hypothalamus of the monkey, an intense AChE activity was observed within the neuronal somata of nuclei supraopticus and paraventricularis. In the paraventricular nucleus, the intensely-stained AChE perikarya are of medium size and have numerous AChE-containing processes. Weakly reactive neurons of smaller size are also present within the paraventricular nucleus. In contrast, most perikarya of the supraoptic nucleus are intensely stained for AChE but their processes are devoid of the enzyme. Other intensely-stained AChE neurons were found in the lateral and perifornical areas and most particularly in the dorsal hypothalamic area. These neurons are of medium size and mostly multipolar. Moderately-stained neuronal somata were encountered in the lateral, dorsal and supra-mammillary areas. Most of the mammillary body neurons, however, are weakly reactive. Such a feature differs from what is seen in the rat hypothalamus where most mammillary body neurons stain either moderately or intensely for AChE (Parent and Butcher, Anat. Rec., 184: 496, 1976). As it is the case in the rat, however, the median eminence as well as most neurons of the ventromedial nucleus of the monkey are virtually devoid of AChE. (Supported by the Medical Research Council of Canada).



FIGURES:

AChE-containing neurons in the bed nucleus of the anterior commissure (Fig A) and in nucleus paraventricularis hypothalami (Fig B) of a DFP-treated monkey (0.2 mg/kg, 10 hours). Bar scale = 100µm

525 IMPAIRED INCIDENTAL PLACE LEARNING IN FORNICOTOMIZED RATS. M. Pisa (SPON: R. Hirsh). Dept. Psychology, McGill Univ., Montreal, Quebec, Canada.

The question was asked whether fornicotomized animals can effectively locate a place on the basis of incidentally acquired information. Food deprived sham operated (CON) and fimbria-fornix lesioned (FF) subjects (Ss) were tested in an H-shaped maze with four goal boxes. Two of the goal boxes were very different from each other and from the alleys of the maze (salient goal boxes) and the two other goal boxes were not (non salient goal boxes). After a series of preliminary sessions in which Ss could explore the maze extensively, eight test sessions followed. In each test session S was first allowed to explore the maze. At the end of the exploratory period S was placed into one of the goal boxes and fed. A test trial followed in which S was put into the start box and allowed to run until it entered the baited goal box. Over the eight sessions the bait was put twice in each of the salient goal boxes and twice in each of the non salient goal boxes. The main finding was that FF Ss made significantly more errors than CON Ss during the test trials although they showed an improvement in the later trials when salient goal boxes were baited. During the exploratory periods, CON Ss shifted preference towards the goal box baited the previous day significantly more than FF Ss. These results suggest that fornicotomized rats are impaired in incidental learning or at least in efficient retrieval of information about place location.

526 EFFECTS OF VAGAL VOLLEYS ON UNIT ACTIVITY OF AMYGDALA, HIPPOCAMPUS, AND CORPUS STRIATUM IN SQUIRREL MONKEYS (SAIMIRI SCIUREUS).
Richard J. Radna* and Paul D. MacLean. NIMH, Bethesda, Md. 20014.

This study represents a continuing investigation of visceral inputs to respective parts of the forebrain. Squirrel monkeys are chronically prepared with a stereotaxic platform for recording extracellular potentials with glass micropipettes and exploring the putamen, amygdala, and hippocampus. The animals sit gently restrained in a special chair. Vagal shocks are applied with electrodes implanted in the jugular fossa. Control stimuli are applied through cervical subcutaneous electrodes.

A highly flexible computer system (CAUDAR) developed for this study substitutes wave-form recognition for a window discriminator and utilizes high-speed graphic display of unit activity, surface plots of potentials of more than one unit, time-interval histograms, and print-out of the statistical analysis.

The records of 158 units proved satisfactory for extensive analysis. Vagal volleys elicited statistically significant responses in 28% of 46 hippocampal units; 12% of 33 in the amygdala; and 24% of 53 in the putamen. Control stimuli affected the activity of less than 1% of the vagally activated units. For each structure there was approximately a one-to-one ratio of initially excited and initially inhibited units. As opposed to previous findings on medial thalamic structures and the mid-line supracallosal cortex, there were no units that responded at regular and relatively short latencies with one to three spikes. Rather, most of the initially excited units in each structure fell into the class of so-called Type 2 units, characterized by discharge of three or more spikes at widely variable and relatively long latencies. The latencies for such units in the hippocampus ranged from 80 to 800 msec. One unit in the central nucleus of the amygdala was of particular interest because of its regular response to vagal volleys with a burst of 8-12 spikes. About one-third of the responsive units of the putamen were distinctive because of complex alternating phases of excitation and inhibition. The CAUDAR system was particularly useful in the statistical analysis of questionably responding units, as well as those showing fall-off in spike height with a rapid rate of discharge.

The findings are discussed in the light of recent evidence implicating dorsal pontine structures in the transmission of visceral information.

527 ELECTRICAL SELF-STIMULATION IN THE RAT: IS THE PRIMING EFFECT DUE TO AN AVERSIVE AFTEREFFECT? Peter Shizgal*
(SPON: Z. Amit) Dept. Psych. U. of Penn. Phila, Pa., 19174

A technique was developed for measuring the aversive aftereffect produced by electrical stimulation at sites in the rat diencephalon that support both self-stimulation and stimulation-escape. The latency to escape a fixed electrical stimulus was lower on trials that were preceeded by signalled, inescapable, stimulation than on trials that were preceeded by the signal alone. It appeared that a slowly decaying aftereffect of the pretrial stimulation summated with the aversive effect of the escapable stimulation and that the magnitude of the aversive aftereffect was reflected in the degree to which pretrial stimulation reduced the subsequent escape latency.

Aversive aftereffects play an important role in some theories of self-stimulation. These aftereffects have been proposed as the cause of the priming effect - the potentiation of subsequent performance for a brain stimulation reward that is produced by pretrial stimulation. Alternatively, aversive aftereffects may be independent of or antagonistic to the priming effect. Just as two different effects, reward and aversion, may be produced while stimulation is on, two different and perhaps antagonistic states may persist following stimulation offset.

The role of aversive aftereffects in self-stimulation was evaluated by separately measuring the priming effect and the aversive aftereffect produced by a given train of stimulation. According to the first theory, these aftereffects are isomorphic while according to the second, they are different and perhaps antagonistic. The priming effect was assumed to be reflected in the reduction caused by pretrial stimulation in the latency to press a lever that delivered a brain stimulation reward. The aversive aftereffect was assumed to be reflected in the reduction caused by the same pretrial stimulation in the latency to press a lever that turned off an automatically initiated train of stimulation. Pretrial stimulation was delivered in bursts of pulses separated by intervals of no stimulation. When the duration of the bursts was increased with the inter-burst interval held constant, the aversive aftereffect increased monotonically. In contrast, the priming effect of the same stimulation first increased and then decreased. Thus, initial results were most consistent with the second theory; priming effects were attenuated by a manipulation (increasing the burst duration from moderate to high values) that increased the aversive aftereffect. However, at some placements, procedural problems in measuring the two aftereffects preclude drawing strong inferences from the findings of this ongoing experiment. Pilot data suggest that these problems can be eliminated by changing the responses required to initiate and terminate stimulation.

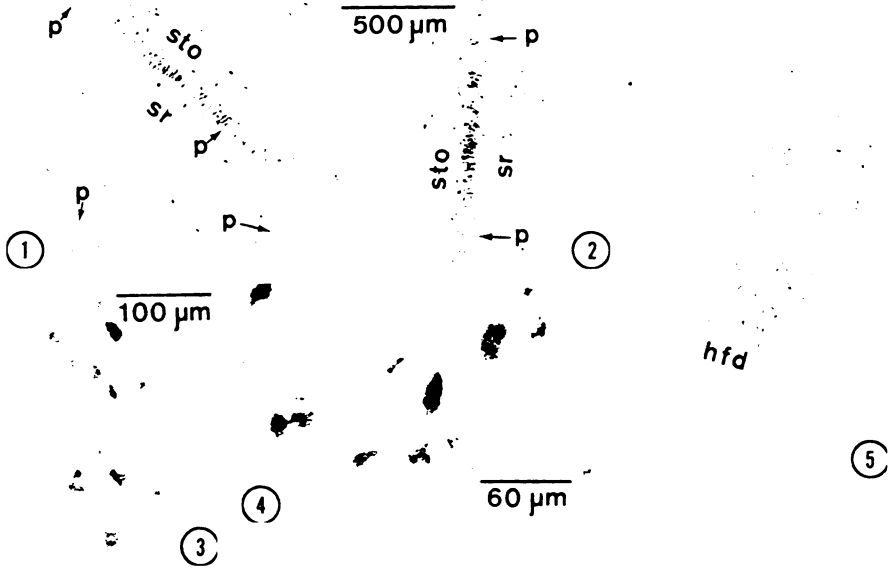
528 DISCOVERY AND MORPHOLOGICAL CLARIFICATION OF ACETYLCHOLINESTERASE (AChE, EC 3.1.1.7)-CONTAINING NEURONS IN THE HIPPOCAMPAL FORMATION OF ADULT RATS. Konrad Talbot[1] and Larry L. Butcher[2]. Department of Psychology (1,2) and Brain Research Institute (2), University of California, Los Angeles, CA 90024, U.S.A.

Application of the pharmaco-histochemical technique for AChE (Butcher *et al.*, 1975, *J. Neural Trans.* 37, 127), which allows preferential demonstration of somatal, as opposed to neuropil enzyme activity, reveals the presence at the light microscopic level of previously undetected AChE neurons in the hippocampus (H) of the adult rat. Utilization of the pharmaco-histochemical protocol also allows clarification of the morphology and detailed distribution of commonly known AChE-containing neurons throughout the adult rat hippocampal formation.

The present study demonstrates neuronal somata containing AChE in *1)* a very limited portion of the hippocampal pyramidal layer, specifically corresponding to CA1c of Lorente de Nó (1934-35, *J. Psychol. Neurol. (Leipzig)* 46, 113) and probably the immediately adjacent CA1b and CA2 of the same investigator (figs. 1 *(dorsal H)* and 2 *(crus of H)*, coronal sections), *2)* the stratum lacunosum (fig. 3) of the regio superior, as well as to some extent the adjoining portions of the stratum radiatum (sr) and stratum moleculare, and *3)* the stratum lucidum of the regio inferior and the sr (figs. 1,2) of the regio inferior. Mellgren (1973, *Z. Zellforsch.* 141, 375) has previously demonstrated AChE neurons in the sr of the regio inferior in immature rats.

Although Shute and Lewis (1966, *Z. Zellforsch.* 69, 334) note a small amount of AChE in somata of rat hippocampal pyramidal cells, they do not report the relatively high enzyme activity of CA1c cell bodies. The failure of large septal lesions to abolish the AChE activity associated with CA1c, as shown in figs. 1 and 2, strongly suggests that the enzyme is present within somata in CA1c.

Consistent with previous reports in the adult (e.g., Storm Mathisen and Blackstad, 1964, *Acta Anat.* 56, 216; Shute and Lewis, 1966, *op. cit.*) and immature (Mellgren, 1973, *op. cit.*; Matthews *et al.*, 1974, *Dev. Biol.* 36, 130) rat, we have also observed some AChE neurons in the stratum oriens (sto) of the H (figs. 1,2,4) and many AChE neurons in the hilus of the fascia dentata (hfd, fig. 5). (Support: USPHS NS-10928 and Scott Fund)



529 DELTA-9-TETRAHYDROCANNABINOL AND THE HIPPOCAMPUS: PARAMETRIC FEATURES OF THE EFFECTS ON CA1 FIELD POTENTIALS. Richard M. Vardaris and Donald J. Weisz* Dept. Psych., Kent State University, Kent, Oh. 44242, and Timothy J. Teyler, Dept. Psych. Soc. Rel., Harvard University, Cambridge, Mass. 02138.

There have been relatively few studies concerning the effects of cannabinoids on the electrophysiology of specific CNS structures. It was found that Δ^8- and Δ^9-tetrahydrocannabinols (THCs) enhance the amplitudes of both short and long latency cortico-cortical evoked responses in postarcuate polysensory cortex of encephale isole squirrel monkeys. In rats deeply anesthetized with urethane, the natural cannabis Sativa compounds, cannabidiol, cannabinol, Δ^9- and Δ^8- tetrahydrocannabinol, had no effect on a hippocampal evoked response although they are reduced susceptibility to hippocampal seizures induced by afferent stimulation. Similar anti-convulsant properties have been observed by other investigators.

The effects of Δ^9-tetrahydrocannabinol on ortho- and antidromically elicited CA1 field potentials were observed in locally anesthetized rats. The drug augmented amplitudes of population EPSP's and antidromic population spikes from pyramidal cells. Latencies to peak amplitude of these responses were increased. Conditioning-test shock experiments revealed that the drug also depressed recurrent inhibition probably mediated by basket cells. It was concluded that Δ^9-THC enhanced postsynaptic excitability and produced disinhibition in the CA1 subfield. The purpose of the present investigation was to characterize the parameters of these effects by observing them for a range of doses, times after injection, and conditioning-test (C-T) shock intervals.

The intact hippocampal preparation utilized unanesthetized paralyzed rats with procainized wound margins and pressure points. Extracellular population responses of the CA1 pyramids were elicited by stimulation of the Schaffer collateral branch of the CA3 axons. Input-output (IO) functions were obtained with symmetrical double-shock stimuli. Then 0, 1, 2, 4, 8, or 16 mg/kg Δ^9-THC was administered IP as an aqueous suspension in the presence of polyvinylpyrrolidone (PVP). Post-drug IO functions were obtained 15, 30, 45, 60, 90, 120, and 150 minutes after injection. Just after each IO function aC-T curve for recurrent inhibition was obtained using intervals of 20, 30, 40, 50, 60, 80, 100, 120, 140, and 160 msec.

As in the prior study Δ^9-THC enhanced amplitudes of the extracellular population spikes and attenuated recurrent inhibition. The dose-effect curve for CA1 spike amplitude was maximal at 4 mg/kg, with doses at 1, 8, and 16 mg/kg showing less effect. Enhancement of population spikes was still increasing 150 min. after injection. Drug effects on the C-T interval function were most apparent for all doses 120 and 150 min. after injection, when inhibition of T-spike amplitudes was significantly reduced at C-T intervals of 60, 80, and 100 msec. Disinhibition was a positive, essentially linear function of dose at 120 and 150 min. after injection. Nonmonotonic functions were obtained for the shorter post-injection delays. It was concluded that the results confirmed those of our prior study and in addition indicate that the peak drug effects require at least 150 min. to develop. Enhancement of postsynaptic excitation reached a peak significantly earlier than disinhibition.

530 HABITUATION OF AROUSAL IN RATS WITH LESIONS OF HIPPOCAMPAL CONNECTIONS.
Phyllis G. Wacker* and Phillip J. Best. Dept. of Psychology, University of Virginia, Charlottesville, Virginia 22901.

Damage to hippocampal connections in the rat is accompanied by a wide variety of changes in behavior. Recent work in this laboratory found hippocampal units to respond more drastically during arousal from slow wave sleep (SWS) by tonal stimuli than following any other manipulation. This arousal habituates over time. Further work found that fimbria-fornix lesions enhance the duration of arousal from SWS but do not drastically modify habituation of this response.

The current study investigates whether the arousal reaction to tones previously paired with footshock will be more resistant to habituation than the arousal reaction to non-paired tones, and whether disruption of the fimbria-fornix or entorhinal pathways has any effect on these arousal reactions and their habituation.

Thirty-six male Sprague-Dawley derived rats were randomly assigned to three groups of twelve animals each. One group received electrolytic lesions of the fimbria-fornix (FF), another group received bilateral electrolytic lesions of the entorhinal cortex (EC), and control group (C) received no lesions.

In all subjects EEG activity was recorded from neocortex and hippocampus. The two tones used were 1 sec duration, 2.9 KHz and 4.5 KHz. For each subject one tone was paired with footshock and the other was not. Each animal was tested for 6 consecutive days. The three groups (FF, EC and C) were each divided into 3 subgroups. On Days 1 and 2 one subgroup received 6 presentations of one tone followed immediately by footshock (.8ma, .5sec), at 15-30 minute intervals. On Days 3 and 4 the paired tone was presented 10 times in a habituation procedure. Each tone presentation was given following at least five minutes of sleep with a final one minute of SWS. Arousal was assessed on the basis of the animal's behavior and neocortical EEG activation. Arousal duration was measured from the onset of the tone until the animal returned to SWS. On Days 5 and 6 the non-paired tone was habituated in a similar manner. The second subgroup had the same procedure except that habituation of the non-paired tone preceeded tone-shock pairings. The third subgroup had tone-shock pairings on Days 1 and 2, habituation to the non-paired tone on Days 3 and 4 and habituation to the previously paired tone on Days 5 and 6.

The data were analyzed by comparing arousal durations for blocks of 5 tone presentations. Both the FF and the EC groups showed significantly longer initial arousal reactions during habituation of the non-paired tone than the Control group (both $p<.05$). There was no difference between the FF and EC groups. The EC group continued to show increased arousal at a level significantly higher than the C group for all four blocks of trials.

When the tone previously paired with footshock was habituated prior to exposure to the non-paired tone there was no difference between groups.

The data indicate that lesions of the fimbria-fornix result in increased arousability from SWS to a tonal stimulus. These lesions play no role in the animal's ability to habituate to this same tone. Animals with entorhinal cortex lesions also show increased arousability from SWS and in addition do not habituate as rapidly or to a level as low as fimbria-fornix lesioned and control animals.

Neither FF or EC animals showed a difference in arousability between a tone that had been previously paired with footshock and a non-paired tone, as did the C group. This indicates the disruption of some influence important in establishing a tone as a conditioned stimulus.

531 THE EFFECT OF FORNICOTOMY ON THE PUNISHMENT OF RUNWAY RESPONSES IN THE RAT. H. Anchel*, H. Okaiche*, H. Barbaree* and A. H. Black. (SPON: J.Diamond) Dept. Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

Four groups of rats were employed. All rats were first trained to run for food reinforcement. Then two groups (one with fornical lesions and one control) were shocked in the goal box when they attempted to eat. The other two groups (one with fornical lesions and one control) were shocked in the runway proper just before entering the goal box.

The results indicated that (1) rats with fornical lesions hesitated primarily when they were near the stimuli in the presence of which they had been shocked, (2) for animals shocked in the goal box, controls showed significantly more hesitation in the start box and runway than rats with fornical lesions, and the same amount of hesitation in the goal box, (3) for rats shocked in the runway, controls showed more hesitation in all locations.

These results were interpreted as indicating that rats with fornical lesions are capable of inhibiting responses when they are near cues in the presence of which the response was punished, and that they differ from controls in terms of the generalization of the inhibition to places other than the ones in which they had been shocked.

532 CORTICOFUGAL FIBER DEGENERATION FOLLOWING LESIONS IN THE MEDIAL PREFRONTAL CORTEX OF MACACA MULATTA. J. Astruc, G. R. Leichnetz and J. T. Povlishock* Department of Anatomy, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia 23298.

Unilateral partial ablations were made on the medial aspect (Walker's Areas 9 and 10) of the prefrontal granular cortex in ten adult rhesus monkeys. Following survival periods of 7-25 days, the monkeys were sacrificed by perfusion with normal saline followed by 10% formalin. The brains were removed and later processed with both Nauta-Gygax and Fink-Heimer silver techniques. Fiber degeneration could be traced through the superior frontooccipital fasciculus to the parietal cortex, and through the uncinate fascicle to the insular cortex, inferior temporal gyrus and entorhinal area. Fiber degeneration was followed through the cingulum into the cingulate and retrosplenial cortices, and entorhinal area. Some degenerating fibers joined both the alvear and perforant paths to enter the hippocampus. Large numbers of corticofugal fibers entered the rostrum and genu of the corpus callosum to cross into the contralateral hemisphere. Subcortical projections, in addition to those to the striatum, were followed to the mediodorsal thalamic nucleus (MDpc) and the anterior thalamic nucleus via the anterior thalamic radiations (rostral inferior thalamic peduncle). Some of these fibers entered and followed the stria terminalis throughout its course, while others left it to traverse the dorsolateral internal capsule to enter the medullary laminae of the globus pallidus, and descended to reach the basal forebrain and amygdala. Finally a small and more diffuse group of fibers coursed caudally with the medial forebrain bundle, traversing the lateral hypothalamus. Some of this group turned laterally, parallel with the ansa peduncularis, traversing the substantia innominata, to enter the amygdaloid complex. The possible significance of prefronio-limbic interconnectivity will be discussed.

533 VISUAL RECEPTIVE PROPERTIES OF HUMAN LIMBIC NEURONS. Thomas L. Babb*, Eric Halgren* and Paul H. Crandall. Brain Research Institute, UCLA, Los Angeles, CA. 90024.

In non-human primates, it has been shown that many areas of the limbic system (especially posterior hippocampal and para-hippocampal regions) receive projections from visual areas such as the lateral geniculate, striate cortex (Casey et al. J. Neurophysiol., 1965, 28:1118-1131) and the inferior pulvinar (MacLean and Creswell, J. Comp. Neurol., 1970, 138:265-278). In order to determine whether visual afferents are relayed to human limbic neurons, extracellular action potentials were recorded from fine wires chronically-implanted in temporal lobe epileptics (Babb and Crandall, Electroenceph. clin. Neurophysiol., 1976, 40:225-243) during a variety of changes in diffuse retinal illumination. Binaural clicks were presented to test for the presence of sensory convergence in human limbic neurons. Single and multi-unit discharges were recorded from over 150 different electrodes in 18 different patients. Neurons in posterior gyrus hippocampi were more responsive to flashes than neurons in the pes hippocampi (Ammon's horn and dentate gyrus). The responses were usually an increase in firing rate about 80 msec. after the flash; however several neurons exhibited decreased firing at that latency. Several neurons in the posterior gyrus hippocampi exhibited sustained firing changes during sustained retinal illumination; however most neurons were only phasically-responsive to changes in illumination. Photic responses of neurons in the amygdala were rarely encountered. Neuronal responses to binaural clicks were not evident. These results indicate that visual projections to human limbic areas probably follow pathways through retrosplenial and lingual cortex to distribute predominantly in posterior limbic cortices.

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534 HIPPOCAMPAL SUBFIELDS AND INHIBITORY AVOIDANCE BEHAVIOR IN MICE. Carl A. Boast*, E.L. Cole*, and S.F. Zornetzer (SPON:J.B. Munson), Dept. Neurosci., Coll. Med., U of Florida, Gainesville FL 32610.

Lesions of the hippocampus can result in behavioral alterations. In attempting to delineate the contribution of hippocampal subfields to these behaviors we have reported that discrete manipulations of the dentate gyrus of the mouse can result in impaired inhibitory avoidance behavior 24hr after training. Lesions of the CA1 subfield will impair performance in this task 15 min but not 24hr after training. Thus, different hippocampal subfields can differentially contribute to inhibitory avoidance.

To further elucidate the role of various hippocampal subfields in inhibitory avoidance behavior, electrodes (125μm diam) were implanted in Swiss/ICR mice 10 days prior to training in such a task. Mice with electrodes located bilaterally in the dentate gyrus or the CA3 region of the dorsal hippocampus were impaired when tested for retention 24hr after training. Mice with bilateral CA1 electrodes or with asymmetric placements were not impaired on the retention test.

Thus, CA1 was the only subfield of the mouse dorsal hippocampus that did not result in a behavioral deficit following minor mechanical trauma. For this reason we felt that the CA1 region could be studied with post-trial electrical stimulation. In a second experiment each mouse received a single pulse (750μA, 1msec) bilaterally in CA1 immediately after training. In the absence of seizures single pulse stimulation of the CA1 subfield does not result in impaired inhibitory avoidance behavior tested 24hr after training.

We suggest that further study of the contribution of dorsal hippocampal subfields to behavior should not be continued using the mouse as an experimental animal.

535 DEVELOPMENT OF HIPPOCAMPAL REWARD THROUGH REPEATED DAILY ELECTRICAL STIMULATION (KINDLING). Kenneth A. Campbell*, N. W. Milgram and Jon K. Christoff*. Dept. Psychology, University of Toronto, Scarborough College, West Hill, Ontario, Canada.

Rates of acquisition of bar pressing for hippocampal stimulation were studied in two groups of rats, one of which had previously received a program of daily hippocampal stimuli over 22 consecutive days, resulting in a gradual progression of convulsive activity. Other work has shown that the neurophysiological substrate of this development of motor seizures is an apparently permanent potentiation of neural transmission (potentiation of evoked potentials: Racine, Gartner & Burnham, 1972). In the absence of stimulation pretreatment and consistent with our previous experience, acquisition of hippocampal self-stimulation was slow: 13 of the 15 control animals required a minimum of 8 daily half-hour testing sessions to acquire the bar pressing response. In marked contrast, 10 of the 15 prestimulated animals learned to bar press within 3 sessions. No differences were found between the two groups in latency to the first bar press, indicating that operant rate was unaffected by the stimulation pretreatment. This suggests that the stimulation pretreatment facilitated response acquisition by affecting the reinforcing value of the hippocampal stimulation. The present findings are interpreted as indicating that electrical stimulation of the hippocampus is not initially reinforcing, but that reinforcing properties will develop as propagation of hippocampal stimulation is potentiated over the course of the stimulation pretreatment.

536 THE ORIGIN OF GUDDEN'S FASCICULUS OF THE POSTCOMMISSURAL FORNIX. R. B. Chronister, R. W. Sikes* and L. E. White, Jr. Neuroscience Research and Department of Psychology, Univ. of South Alabama, Mobile, AL 36688

Recently, using retrograde transport methods, we determined the origin of the direct hippocampo-anterior thalamic bundle in the rat. This bundle was seen to arise from specialized areas of the subiculum and presubiculum. Furthermore, it became obvious that the cells of origin could be characterized in the HRP preparations and comparisons made with Nissl and rapid Golgi impregnations. The cell bodies of origin are in the perialvear subiculum and in the deep layers of presubiculum. The remaining subicular projection systems seem to emanate from the other regions of the subiculum and presubiculum. For example, the inner layers of subiculum (toward the molecular layer) seem to project to the septal region. The outer layers of presubiculum seem to contribute to the perforant path. Therefore, the cell bodies within these structures have a precise localization depending upon their projections.

The nature of the inputs to these areas also show cellular segregation. The cingulum bundle and amygdala-hippocampal efferents project to the respective presubicular and subicular areas. Cingulum efferents project to the external presubiculum and not the deep layers, thus giving a discrete input-output relationship to presubiculum. In addition, careful comparison of anterograde and retrograde results allows typing of cells for further analyses of dendritic and axonal profiles, and synaptic interrelationships.

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537 RABBIT HIPPOCAMPAL UNIT-SLOW WAVE PHASE RELATIONS. Peter Coyle. Dept. of Anat., Sch. Med., Univ. Mich., Ann Arbor, MI 48109

Analog demonstrations suggested nonrandom phase relations exist between hippocampal units and the theta rhythm regular waves during certain behaviors or under drugged conditions. That nonrandom unit-regular slow wave phase relations exist and depend upon midbrain and more caudal ascending fiber systems was investigated. Midbrain transected rabbits receiving eserine (0.30 mg/kg body wt. I.V.) were sampled. Units were recorded with stainless steel microelectrodes. Recording sites were marked. Regular slow waves were filtered from the microelectrode. Occurrence times of units and concurrent slow wave cycle durations were measured with a Prime 200 Digital Computer. Unit-slow wave phasegrams were constructed for 47 cells and compared to a random phase model using a chi-square test for evaluation of independence of distributions. Rejection of the null hypothesis at high confidence levels ($p \leq 0.01$) indicated the neuronal data differed from the random model. A graded array of phasegrams was found. Filtered and shuffled data phasegrams provided evidence unit-slow wave phase relations were stable during the sampling period and with slow wave frequency changes. High confidence level phasegrams were not the result of chance occurrence of units for unit and slow wave signal nonstationarities existed in the time domain. (Supported by Univ. Mich. H. H. Rackham Grant & Fellowship, and Med. Sch. Gen. Res. Support Funds)

538 DESCENDING PROJECTIONS OF THE MAMMILLARY NUCLEI OF THE RAT. Judith A.F. Cruce, Department of Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

The efferent projections of the mammillary nuclei of the rat were studied after injections of either tritiated leucine or tritiated proline stereotaxically aimed at the medial or lateral mammillary nucleus. The volume of the injection ranged from .03 to .4 µl containing 1.8-8 µCi. Survival times ranged from one hour to one week. Brains were sectioned on a freezing microtome and processed by standard autoradiographic methods. Axons leave the mammillary nuclei as the principal mammillary tract which bifurcates into the ascending mammillothalamic tract (MTT) and the descending mammillotegmental tract (MTG). While the MTT forms a compact bundle throughout its diencephalic course, the MTG is composed of many fascicles which are spread in a wide dorsal-ventral extent throughout the mesencephalon. The labeled MTG fibers are best visualized in brains cut in the sagittal plane; they intermingle with and pass at right angles to the decussating fibers of the superior cerebellar peduncle. Major projection sites of MTG include 1) dorsal tegmental nucleus (DTN), 2) ventral tegmental nucleus (VTN) and 3) pons. Labeled fibers first pass to the more rostrally located VTN, then through the medial longitudinal fasciculus into the DTN; dense label is present around the neurons of both VTN and DTN. The pontine projection site forms a narrow medial strip. At the rostral edge of the pons label is present within the pontine grey; at more caudal levels the labeled area is more dorsal, so that the label comes to lie within the pontine tegmental reticular nucleus (RTP) located immediately dorsal to the medial lemniscus. Thus the pontine label forms a continuous band from the rostral-ventral pontine grey to the more caudal-dorsal RTP. Those areas of the pons which are labeled are not cytoarchitectonically distinct from immediately adjacent areas which are not labeled. Grant support NIH 5 PO1-NS-06225, NSF 17835, JHU M.11.2096.

539 BEHAVIORAL AND ANATOMICAL CHANGES FOLLOWING TRANSECTIONS OF LIMBO-THALAMIC FIBERS. Robert E. Davis and Ernest W. Kent, University of Illinois at Chicago. Dept. of Psychology.

Anatomical and neurophysiological evidence suggest that the anterior thalamic nuclei, (Atn) interface limbic (hippocampus, septum) and brain stem (reticular formation, mammillary bodies) structures to medial cortical areas. To clarify this relation we examined the behavioral and anatomical changes resulting from interruptions of fibers passing medially at the septo-thalamic border. Transections were produced in a variety of coronal planes extending anteriorly to an area rostral to the columns of the fornix, posteriorly to the stria medullares thalami, and medial-laterally covering an area 1.5mm on either side of the midline. Behaviors studied following knife cuts were acquisition of bidirectional avoidance (100 trials/day over 2 days; 14 days postoperatively), and closed-field activity (30 min/day; days 1-28). Anatomical changes were identified by comparison of control and transected animals following injections of horseradish peroxidase (.2µl; 1-10%) into the Atn.

The anteriorly placed cuts yielded changes similar to those found following septal lesions; facilitated acquisition of bidirectional avoidance and initial hyperactivity which habituated within a session. These cuts eliminated retrograde transport of Hrp to the medial septal nuclei and decreased the labeling of the lateral septal nuclei; no other changes were noted. Transections posterior to the columns of the fornix, not affecting the stria medullares, produced a behavior syndrome similar to that seen following hippocampal destruction; facilitated active avoidance and hyperactivity which does not habituate within a session. The distribution of labeling after these cuts was similar to the anterior transection with the additional observation that Hrp graules are no longer present in the subiculum-presubiclum areas.

540 RESPONSES OF THETA CELLS IN RABBIT HIPPOCAMPUS TO SENSORY STIMULI PRESENTED DURING MOVEMENT AND NON-MOVEMENT IN A TREADMILL. P.M. Di Lorenzo* and J.S. Schwartzbaum* (SPON: V. Laties). Univ. of Rochester, Rochester, N.Y. 14627.

Single unit activity was recorded from dorsal hippocampus of freely-moving rabbits in a treadmill to examine a) functional differentiations among theta cells, and b) significance of background theta patterns of activity with respect to unit response to sensory stimuli. Repetitive 0.5 sec. click or flash stimuli were presented during stationary behavior and enforced hopping movement (detected by a noisy lead). Unit data were analyzed by means of spike-interval, cluster-interval and post-stimulus histograms. Theta cells, defined with respect to periodicity of discharge during enforced movement, could be functionally differentiated in terms of inhibitory-excititory patterns of response to sensory stimuli during non-movement. Preliminary data also suggested differences in persistence of theta activity during non-movement. All theta cells that responded to sensory stimuli during non-movement (in the absence of ongoing theta activity) showed no response to the same stimuli during movement (and associated theta activity). Similar effects could be demonstrated with irregularly spaced sensory stimuli presented during non-movement and movement. While theta cells may not display invariant reaction to neutral sensory stimuli during non-movement, their patterns of reaction to such stimuli clearly interact with movement conditions and associated differences in background theta-nontheta modes of activity.

541 CUE ELIMINATION EFFECTS ON DISCRIMINATION BEHAVIOR OF RATS WITH SEPTAL LESIONS. Peter J. Donovick, Richard G. Burright*, Roger D. Sikorszky*, Nicholas J. Stamato*, and William W. MacLaughlin*. Dept. Psychol., SUNY, Binghamton, N.Y. 13901.

Over the past several years we have conducted a series of experiments to elucidate the factors controlling the acquisition of a brightness discrimination by rats with septal lesions and their surgical control counterparts. We found that the behavior of the two groups was differentially effected by both the experience with relevant cues and the amount of experience with the task. Further, the specific response measure employed was critical to detecting group differences.

In the present series of three experiments we tested control and septal lesioned rats in problems designed to: (1) examine the rate and mode of learning a task with multiple solutions; and (2) the effects of eliminating potential solution cues after criterion was reached. Rats with septal lesions learned the task using the "simplest solution" and indeed were faster than control animals in original learning. Cue elimination differentially altered behavior of the two groups depending upon which strategy was subsequently available to the animal. These results were interpreted as being consistent with the hypothesis that septal lesions alter the relative weighing of important environmental cues.

542 SEPTAL STIMULATION,LIGHT AVERSION AND VISUAL EVOKED POTENTIALS IN THE RAT. Robert S. Dyer, Patrick Eacho*,Paul G. Jenko* and David S. Olton. Dept. Environ. Med. & Dept. Psychol., Johns Hopkins Univ. Baltimore, MD 21205.

The septal area may modulate reactivity to visual stimuli. Lesions in the septal nuclei increase rat light aversion scores, behavioral reactivity to flashes and amplitudes of flash evoked potentials (VEP's) recorded from cortex. It has been argued that lesion induced changes in behavior are dissociable from lesion induced changes in VEP's (JCPP,'72). The present study examined the influence of septal stimulation in rats upon light aversion as measured by the Olton technique (P&B,'74) and upon VEP's. Bipolar stimulating electrodes were implanted in the septal area and skull screws were implanted over the visual cortex in 15 rats. After recovery (1 wk), rats were placed in a recording chamber. Recording sessions were arranged so that each animal received 7 stim (300ua,0.2ms,100hz biphasic pulse trains 100ms long) alternated with 7 nonstim trials. Each trial consisted of the average response to 25 light flashes, with stimulation ending 75 ms before the flash. Following the recordings, animals were placed in the light aversion apparatus, where they were either stimulated as above at 1 hz or not stimulated according to a predetermined schedule. Time spent in light and dark boxes was compared for stim and nonstim blocks of time. Medial septal stimulation increased VEP amplitudes, but ventrolateral septal stimulation decreased amplitudes. No clear effect of stimulation upon light aversion behavior was found. These experiments may be taken as supporting evidence that there is a septal influence upon the visual system which is independent of any influence upon behavioral measures of light aversion. Similar electrophysiological data have been observed by others in cats (EN,'71). The septal influence upon the visual system may be mediated by the septotectal tract. Preliminary results suggest that superior colliculus lesions attenuate the effect of septal stim.

543 THE ROLE OF THE MAMMILLARY EFFERENT SYSTEM IN SPATIAL MEMORY AND AROUSAL. Timothy D. Field‡ Judith Rosenstock‡ E. Cameron King‡ and Ernest Greene. Dept. Psych., USC, Los Angeles, Ca. 90007.

Previous work from this laboratory has shown that output structures of the hippocampal formation are involved in mediation of spatial memory habits. The present experiments indicate that lesion of the medial mammillary nucleus leads to inferior performance on a delayed spatial alternation task by rats, and also to increased activity in exploration of an open-field. Lesion of mammillary efferent pathways, mammillothalamic or mammillotegmental tract, also produces a spatial impairment. These tract lesions have a differential effect upon open-field activity, however, with the mammillotegmental lesion producing hyperactivity and the mammillothalamic lesion rendering the rats very inactive and unresponsive. It is suggested that the mammillary bodies function in the limbic system in mediation of arousal and in short term processing of spatial memories.

544 EFFECTS OF MESENCEPHALIC AND PONTINE BRAIN STEM STIMULATION ON HIPPOCAMPAL NEURONAL ACTIVITY IN CATS. David M. Finch and Thomas L. Babb. Reed Neurological Research Center, University of California, Los Angeles, California 90024

A physiological action of raphe nuclei in the mesencephalon and nucleus locus coeruleus in the pontine tegmentum on hippocampal neuronal activity has been described in the anesthetized rat (Segal, Brain Res., 1975, 94, 115-131, and Segal and Bloom, Brain Res., 1974, 72, 99-114). We recorded hippocampal neuronal activity with micropipettes in acute cats under general anesthesia and in drug-free chronic cats that were painlessly restrained during recording sessions. Hippocampal pyramidal cells were physiologically identified by their characteristic response to fornix stimulation. Extracellular, "quasi-intracellular," intracellular, and field potential responses were recorded in dorsal hippocampus following electrical stimulation (0.5 msec pulses, 1-5 mA) of the pontine tegmentum and of mesencephalic brain stem regions including raphe nuclei. Inhibitory and mixed inhibitory-excitatory neuronal responses were commonly seen in hippocampus. Latencies of responses were variable, from about 10 msec to more than 100 msec, and the durations of responses were sometimes greater than 400 msec. Brief trains (duration, 50 msec) of stimulus pulses applied at 100 Hz were much more effective than single pulses in eliciting neuronal responses in hippocampus. These findings provide evidence that in the cat there are pathways from these brain stem areas to dorsal hippocampus. (Supported by NIH Postdoctoral Fellowship 1 F32 NS05137-01)

545 DIRECT SEPTO-HYPOTHALAMIC PROJECTIONS IN THE RAT: AN AUTORADIOGRAPHIC STUDY. D. R. Garris*, and J. A. Mitchell. Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201.

Axonal projections from neurons located in the medial and lateral septal nuclei (MSN and LSN) were traced autoradiographically following a 20 nl injection of tritiated leucine (400μCi) into either nucleus. The animals were perfused 48 hrs. post-injection, the brains removed, processed for paraffin embedding, sectioned (17μ), mounted on slides, dipped in Kodak NTB-3 emulsion, exposed for 14-21 days (at 4°C) and developed. Silver grains overlying hypothalamic nuclei were counted using bright field microscopy.

The MSN projected bilaterally via a midline route to the diagonal band of Broca (DBB), the pre-optic area (POA), the suprachiasmatic (SCN), paraventricular (PVN), ventromedial (VMH) and arcuate (ARC) nuclei. In addition, grains were observed in the fibrous zone of the median eminence (ME). Lateral coursing fibers traveled with the medial forebrain bundle (MFB), silver grain aggregations being observed in the supraoptic (SON) and medial mammillary (MMN) nuclei. Projections into the midbrain region were not followed in this study. Grain density indicated that the majority of LSN axons projected to, and terminated in, the MSN. Fibers projected ipsilaterally through the POA, SON, SCN, ARC, ME and MMN with axonal projections observed throughout the MFB.

In summary, the neurons of the MSN project to the medial hypothalamic nuclei via a midline route and through the lateral hypothalamus via the MFB. Both the medial and lateral MSN projections were bilateral. LSN axons projected strongly to the MSN with the remaining fibers coursing along the ipsilateral MFB to terminate in several parvicellular nuclei. These data suggest that a direct septo-hypothalamic pathway exists in the rat. (Supported by NIH Grant RR-05387-13.)

546 FINE STRUCTURE OF CHOLINERGIC HABENULA-INTERPEDUNCULAR TRACT. T. Hattori, V.K. Singh, E.G.McGeer and P.L.McGeer. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Choline acetyltransferase (CAT) has been localized at the light and electronmicroscopic levels in the habenula-interpeduncular tract using the peroxidase-antiperoxidase (PAP) indirect immunohistochemical technique. Using the rabbit anti-human CAT, the cell somata of the medial habenula were strongly and specifically stained for CAT, whereas a more diffuse and faint stain was observed in the lateral habenula. In the interpeduncular nucleus (IP), heavily stained unmyelinated axons and some weakly stained large preterminal boutons (~ 1 μm) could be observed. Boutons were irregularly stained. The positive boutons contained moderately packed large round vesicles (55-65 nm). The outer membranes of the vesicles were usually more intensely stained than the interior. In a few cases, the stained boutons made asymmetrical synaptic contacts with several dendritic elements.

Classification of synapses in the IP has revealed that the morphological type of synapses mentioned above is the most prominent type, accounting for 75-80% of a total of 831 synapses counted. In addition, at least 30% of this type made boutons "en passant" with major dendrites. About 6 - 10% formed a paired (crest) synapse with small dendritic protrusions. In a few cases, this type of bouton was observed to make synaptic triads and reciprocal synapses with the same or other types of boutons. Thus, it can be stated that long axis habenula-IP cholinergic neurons not only have large terminal boutons with large round vesicles, but they may also have much more complicated synpatic arrangements than other known striatal cholinergic system.

547 MOLECULAR LAYER NEURONS IN THE OPOSSUM FASCIA DENTATA. James C. Hazlett and Neil J. Farkas* Dept. Anat., Wayne State Univ., Sch. Med., Detroit, MI 48201

Presently we are utilizing the zinc-chromate modification of the Golgi technique (Fox et al., '51) to impregnate neurons within the molecular layer of the fascia dentate in adult opossums. Our observations of this material have confirmed the presence of the classically described displaced granule cells (Ramon y Cajal, '93) and short-axon neurons (Sala, '91 and Ramon y Cajal, '93). A second variety of short-axon cell possessing multiple axon-like processes and a few scattered dendritic appendages is occasionally observed. In addition two distinct varieties of fusiform neurons are found. The first has a medium sized soma (21μ in diameter) which is always found just above the granule cells. Two sparsely branched dendrites arise from opposite poles of the soma and generally course parallel to the granular layer. Somatic and dendritic spines are occasionally observed and the axons which arise from proximal dendrites pass into the granular layer. The second variety of fusiform neuron is smaller (14μ in diameter) and the dendrites tend to bend "upward" into more superficial portions of the molecular layer. Somatic and dendritic spines are found on some but not all examples of this cell type. The axons arise from proximal dendrites and course transversely in the deeper portion of the molecular layer. Each variety of nerve cell mentioned above (displaced granule cells, short-axon neurons and fusiform cells) possesses dendritic arborizations which appear maximal in the frontal plane. We have observed another variety of neuron with proximal dendrites oriented in the long axis (anterior to posterior) of the fascia dentata. The somas of this type of neuron are medium in size (19μ in diameter) and may give rise to as many as four dendrites. Each dendrite is subsequently cut off in section within 50 micra of the cell body. In several examples an axon initial segment arises from one of the proximal dendrites. (Supported by USPHS Grant No. RR 05384.)

548 A THALAMO-HIPPOCAMPAL CONNECTION IN THE RAT. Miles A. Herkenham* (Spon: W.J.H. Nauta). Psychology Department, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

The nucleus reuniens, a midline thalamic structure lying just above the third ventricle, was the exclusive target of a microelectrophoretic injection of tritiated proline and leucine. The autoradiograms revealed a massive fiber system that ascends to the callosal genu and travels back in the cingulum to the callosal splenium where it turns ventrally and rostrally to enter and massively terminate in a discrete, continuous band in the stratum moleculare and stratum lacunosum of the CA1 fields that abut the hippocampal fissure. Components of this system also innervate layers 1 and 3 of the entorhinal cortex and layer 1 of the cingulate cortex. Still other fibers from reuniens terminate sparsely and diffusely in the medial and lateral preoptic and hypothalamic regions, amygdaloid complex, nucleus accumbens, claustrum, septum, olfactory tubercle and anterior olfactory nucleus, pars dorsalis. A small descending projection passes to the central gray and ventral tegmental area.

These results offer the first evidence of a direct thalamo-hippocampal projection. Injections of horseradish peroxidase (HRP) into the cornu Ammonis caused retrograde cell labeling in the nucleus reuniens and nowhere else in the thalamus. HRP injections in the nucleus reuniens itself labeled a wide variety of brain regions. Consistently labeled were cells in the anteromedial cortex and subiculum. Numerous additional labeled cells were found in many paramedian structures such as the cingulate cortex, septum, preoptic areas, medial and posterior hypothalamus including VMH and the premammillary nuclei, and the central gray substance and raphe nuclei. Labeled cells were also found in the medial amygdala, zona incerta, some medial and deep regions of the pretectum and superior colliculus, and parabrachial regions of the caudal midbrain tegmentum. Supported by PHS Grants NS 06542 and MH 25515 and NIH Fellowship NS 02101.

549 NEURONAL ACTIVITY IN HIPPOCAMPUS DURING A SPATIAL ALTERNATION TASK. A. J. Hill* (SPON: J. Olds). Div. Biol., Calif. Inst. Technology,

Rats are trained in an automated T-maze to perform a left-right alternation for food reward. During training, recordings are made from chronically implanted extracellular electrodes in various brain regions. A miniaturized biotelemeter is used, allowing the animals to move virtually without restriction through the apparatus.

Recordings from dorsal hippocampus indicate that firing of some hippocampal neurons is clearly correlated with the rat's position and orientation in the maze. This response is often quite specific: some units fire when the rat is in a particular part of the maze and facing or moving in a particular direction, but rarely, if ever, fire in other circumstances.

Responses can be made to disappear by changing various aspects of the regions in which the units fire and to reappear by reversing those changes. Units displaying such behavior correspond closely to the "complex cells" described by Ranck (Ranck, Exp. Neurol. 40:461, 1973). The observations can be reconciled with previous indications that the hippocampus may function, at least in part, as a map of the rat's environment (O'Keefe and Dostrovsky, Brain Res. 34:171, 1971).

550 RESPONSE SUPPRESSION ON TWO OPERANT SCHEDULES BY RATS WITH SEPTAL LESIONS. Stephen H. Hobbs. Dept. Psychol., Augusta College, Augusta, Ga. 30904.

Rats with septal lesions, as compared with sham operated or intact controls, are typically found to have increased rates of responding on appetitive tasks, but to exhibit less suppression of such behavior when aversive stimuli are applied. However, we have recently found evidence of considerable suppressive ability by rats sustaining septal damage (Hobbs, IRCS Med. Sci. 3: 354, 1975; Hobbs et al., presented at SEPA, 1976). This was further evaluated in the present two experiments in which animals bar pressed for water on either a variable-interval (VI) schedule of 30 sec or a 15-sec differential schedule of low reinforcement (DRL). In Exp. I (PUN) each response of operated and control rats during the presentation of a 2-min auditory stimulus resulted in delivery of footshock (.18 sec at .1, .3, .5, or .7 ma). In Exp. II (CER), using naive animals, the 2-min auditory stimulus was terminated by a single delivery of footshock (.15 sec at .5, .7, .9, or 1.1 ma). As expected, rats with septal lesions in both experiments had higher response rates than controls on both the VI and DRL schedules. Nevertheless, brain-damaged animals suppressed responding to the subsequent presentations of the auditory stimulus at least as well as controls under each shock intensity in both the PUN and CER experiments. These findings contrast sharply with those of Harvey et al. (JCPP 59: 37, 1965) who reported that rats with septal lesions generally displayed less suppression than control animals on PUN and CER paradigms. The divergent results most probably reflect differences in lesion placements and/or the current use of noncontinuous schedules of reinforcement. It was additionally confirmed, following suppression testing, that operated animals of this study were more reactive to footshock, consumed more water, and were more emotional than their controls.

551 EFFECT OF REVERSAL TRAINING OF VISUAL DISCRIMINATION PROBLEMS ON THETA RHYTHMS FROM THE CAT HIPPOCAMPUS. J. Eric Holmes, Donald Martyn,* and Judith Gelfman.* Department of Neurology, USC School of Medicine, Los Angeles, California 90033

Cats with chronically implanted bipolar EEG electrodes in the dorsal hippocampus were trained in a T maze or in a light-dark discrimination box to food rewards. When performance reached 80% correct for four days, recordings were made, and then the stimuli were reversed and recordings made during the initial learning of the new problem and again at 80% correct responses. Reversal training is difficult for animals with bilateral hippocampal lesions and we assumed that the hippocampus would therefore be involved in whatever neurophysiological mechanisms were used by the animals in this type of learning. Theta rhythms were recorded during all reversal trials in the T maze, which required the cats to walk seven feet to the goal. Theta was also present during more than 50% of reversal trials in the problem box where no walking was involved and the response was opening a plastic panel to the goal. Theta also appeared during the reward period (eating) and at that time the frequency was lower than during approach and choice periods. These findings are compatible with several hypotheses concerning the relationship of hippocampal theta to behavior, and they do support the position that the rhythm occurs when the hippocampus is activated.

552 NEUROPHYSIOLOGICAL CORRELATES OF ACUPUNCTURE. Samuel Jacobs. Dept. Ergonomics, UCSB, Santa Barbara, CA. 93106.

Single unit activity from chronically implanted squirrel monkeys was analyzed to evaluate the effect of acupuncture stimulation on limbic and thalamic structures associated with pain. Considerable data now supports the notion that the perception of both the quality and intensity of pain is not expressed as a predictable aversive response but is strongly influenced by altered neuronal activity in limbic structures affecting mood and behavior (Exp. Neurol., 46:452, 1975). Further, there is clinical evidence (Lancet, 11:564, 1973) suggesting that the efficacy of acupuncture analgesia is strongly dependent on attitudinal factors such as anxiety and culture. Extracellular recordings, therefore, were obtained from n. parafasicularis (PAF) and n. ventralis posteromedialis (VPM) of the thalamus and the lateral septum (LS), basal amygdala (AB) and anterior cingulate cortex (ACi). Single unit activity was obtained prior to and after acupuncture stimulation of points used for diminishing the perception of pain during oral surgery. Baseline and experimental discharge frequencies as well as the interspike interval histograms (ISIH) of both VPM and PAF units remained unchanged and resembled a Poisson process. Limbic activity, however, showed statistically significant changes in both cell firing rate and the ISIH in response to acupuncture stimulation. Finally, in one group of animals subjected to tooth-pulp stimulation, acupuncture proved totally ineffective in raising pain thresholds compared to morphine which increased threshold values 37-51% $\pm$ 2.1. This may be interpreted as a selective response to acupuncture in CNS structures primarily concerned with the affective component of pain.

553 ELECTRICAL STIMULATION OF THE AMYGDALA ALTERS FIRING PATTERNS OF CELLS IN THE SEPTUM. Kenneth J. Kant. Sch. of Nursing, Univ. of Tenn./ Knoxville, Knoxville, TN. 37916.

Earlier work by the author showed that the amygdala has a depressive effect on self-stimulation of the septum in rats. The experiment reported here was performed to determine if such a depressive effect could be exhibited at the cellular level in the septum.

Bipolar electrodes were used to electrically stimulate the amygdala. Tungsten microelectrodes were used to record extracellular action potentials from the septum before, during and after amygdalar stimulation. The same electrodes were also used to record responses in the septum evoked by stimulation in the amygdala.

Cells in the dorsal septal area showed little change in activity with electrical stimulation of the amygdala, but cells in the basal septum normally had one of two kinds of response alteration: a general decrease in firing rate; or, a quick, short-lasting activation followed by a longer period of suppression. The evoked responses obtained were not consistent in their wave form but there seem to be correlations in time between the large negative wave of the evoked response and the increase in cell activity and between the large positive wave of the evoked response and the decrease in firing of the cells.

These data are interpreted to mean that the amygdala does have a direct suppressive effect on certain areas within the septum.

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554 INPUT-OUTPUT RELATIONSHIPS IN THE MAMILLARY BODY NUCLEI. G.K. Kostopoulos* and J.W. Phillis (SPON: A.A. Boulton). Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Sask., Canada S7N OWO.

Although the mamillary bodies (MB) have been linked to a memory function in humans and despite much investigation little is known about their synaptic physiology and role in animal behavior. Constituting one of the major recipients of hippocampal output via the fornix (FO), these nuclei also receive afferents through the mamillary peduncle while their two major efferent paths distribute to anterior thalamus (AT) and ventral tegmentum. In order to investigate the input-output relationships into these nuclei we initiated electrophysiological experiments with cats under barbiturate, and rats under urethane, anaesthesia. Antidromic and orthodromic field potentials as well as unit responses were recorded upon stimulation of AT and different parts of the FO system respectively. About half of the neurons studied within the MB were identified as relay neurons to AT and their spontaneous (usually low) as well as evoked electrical activity was studied. Every one of the mamillothalamic neurons tested in both species (34) responded with a single spike or a burst response to FO stimulation usually followed by an inhibitory period of at least 60 msec duration. The latency for the first spike averaged about 4-6 msec for the rat and 6-8 msec for the cat. Apparently this is a monosynaptic response and the stimulated fibers in the FO belong to neurons of the ipsi and contralateral hippocampal (subiculum) formation. The conduction velocity of the mamillothalamic axons ranged from 2.5 to 25 M/sec and those of the FO between 1.5 and 3.5 M/sec. 80% of the non-identified cells responded in a similar way. Occasional long latency (30-50 msec) excitatory responses to septal stimulation were encountered. The majority of the non-identified MB neurons were excited mono- or polysynaptically by AT stimulation. (Supported by the Medical Research Council of Canada.)

555 PROJECTIONS OF THE ARCHIPALLIUM IN THE PIGEON,COLUMBA LIVIA. Philip F. Krayniak* and Allan Siegel. Depts. Anatomy & Neuroscience, (Spon:H.Brezenoff) N.J. Medical School, Newark, N.J. 07103.

Efferent connections of the archipallium (hippocampal formation) in the pigeon were studied utilizing radioautographic techniques. Separate injections of ^{3}H-Leucine were placed into the region of the medial wall of the avian hemisphere at different levels along its rostrocaudal axis. Differential projections were observed from this region of cortex to the septal area. Fibers from rostral levels of dorsomedial cortex project to both the medial part of the precommissural septum and to the nucleus of the diagonal band. Fibers arising from cells located at central levels of the hemisphere project ipsilaterally to the dorsal septum at the level of the anterior commissure. These fibers, however are also distributed diffusely to more ventral portions of the interomediolateral precommissural septum. Fibers arising from the caudal aspect of the medial wall of the archipallium project ipsilaterally to the dorsolateral part of the postcommissural septum and bilaterally to more central portions of this aspect of septum.Other projections of the hippocampal formation which bypass the septum via the postcommissural fornix in the mammal could not be identified in the present study of the pigeon. Thus, it appears that the projections arising from the medial wall of the hemisphere in the pigeon correspond principally to the precommissural fornix of the mammal.

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556 ALTERED RESPONSIVENESS TO RECEPTOR-STIMULATING DRUGS AFTER HIPPOCAMPAL DAMAGE. Linda P. Lanier* and Robert L. Isaacson. Dept. Psychology, University of Florida, Gainesville, Florida 32610

The effects of hippocampal damage upon catecholaminergic receptor sensitivity were examined by assessing the responsiveness to dopamine (DA) and norepinephrine (NE) receptor stimulating agents. The results indicate that hippocampally damaged animals show a small but significant hyposensitivity to the DA receptor stimulant 1-(2"-pyrimidyl)-4-piperonyl -piperazine (ET-495) one week but not three weeks postoperatively when compared with cortically damaged and unoperated control rats. In addition, rats with hippocampal damage show a significant supersensitivity to the effects of the NE receptor stimulant clonidine which is evident three weeks but not one week postoperatively when compared with normal animals and cortically damaged controls.

557 THE PREFRONTAL-AMYGDALOID PROJECTION IN THE MONKEY: A SILVER AND ULTRASTRUCTURAL STUDY. G. R. Leichnetz, J. T. Povlishock* and J. Astruc. Department of Anatomy, Medical College of Virginia, Richmond, Va. 23298.

Degeneration resulting from unilateral partial ablations of the medial prefrontal cortex in ten rhesus (Macaca mulatta) and three cynomolgus (Macaca fascicularis) monkeys was studied in order to resolve the persistent question of a primate prefronto-amygdaloid projection. The rhesus brains (7-25 day survival) were processed using Nauta-Gygax and Fink-Heimer silver methods, whereas cynomolgus brains (48-72 hr. survival) were processed for electron microscopy. Prefrontal fiber degeneration followed a dorsal and ventral route to the amygdala. In the principal trajectory, the dorsal route, fibers travelled ventromedial to the head of the caudate nucleus, turned dorsally with the anterior thalamic radiations, joined the stria terminalis, and at midthalamic levels turned ventral, traversing the dorsolateral portion of the posterior limb of the internal capsule to enter the medullary laminae of the globus pallidus. While some fibers were given off to the pallidum, and, inferiorly, to the substantia innominata, most descended into the amygdaloid complex and divided to form fibrous laminae outlining the basal amygdaloid nucleus. The silver material suggested that the prefrontal projection was heaviest to the basal amygdaloid nucleus, pars lateralis. However, preterminal and terminal fiber degeneration were also observed in the lateral and central amygdaloid nuclei. Electron microscopy demonstrated both degenerating axons and terminals in the basal nucleus, characterized by their electron density. The degenerating terminals were seen in relation to distal dendritic segments of the large neurons of this nucleus. No degenerating profiles were identified in relation to either the neuronal soma or proximal dendrites. This connection may provide the anatomical substrate for a prefrontal influence in agonistic behavior.

558 PHYSIOLOGICAL IDENTIFICATION AND ANALYSIS OF DENTATE GRANULE CELL RESPONSES TO STIMULATION OF THE MEDIAL AND LATERAL PERFORANT PATHS. B.L. McNaughton* and C.A. Barnes* (SPON: J.C. Fentress). Dept. Psychol., Dalhousie Univ., Halifax, Nova Scotia, Canada, B3H 4J1

Stimulation of the dorsomedial or ventrolateral perforant pathways resulted in qualitatively different extracellularly recorded EPSPs in the fascia dentata of the rat. The two potentials had peak latencies of 4.7 msec and 7.5 msec respectively, and the width at half amplitude of the former was approximately 50% less than the latter. Both potentials were able to follow brief stimulus trains of 100Hz, thus verifying their monosynaptic character. Medially elicited responses presented a peak negativity approximately 100-130 µm deeper in the molecular layer than laterally elicited responses. Stimulation at 200 µm intervals along a dorsomedial to ventrolateral tract in the angular bundle yielded a step function rather than a continuum, of EPSP peak latencies, thus verifying Hjorth-Simonsen's (J. Comp. Neur., 146: 219-232, 1972) demonstration of the separateness of the two pathways. Both pathways were able to induce granule cell discharge, however, laterally elicited spikes were delayed. Stimulation at intermediate locations frequently elicited double spikes from the granule cell population. Both pathways showed facilitation with double stimuli at short intervals, and post tetanic potentiation lasting at least 30 minutes with no significant decrement. Population spikes elicited by either pathway were inhibited for as long as 100 msec after a single discharge.

559 LIMBIC CORTICAL PROJECTIONS TO THE THALAMUS. Richard C. Meibach* and Allan Siegel. Depts. of Anatomy and Neuroscience, N.J. Medical School, Newark, New Jersey 07103.

The purpose of this study was to determine the origin and distribution of fornix fibers which project to the anterior thalamus. ^{3}H-leucine injections were placed into the hippocampus, subicular cortex (SC), and retrosplenial cortex (RS) in the rat. It was observed that fibers from the SC project to the anteromedial (AM), anteroventral (AV), anterodorsal (AD) and laterodorsal (LD) thalamic nuclei. It appeared that only fibers from dorsal and posterior levels of the SC contribute to this pathway. No projection to the thalamus was observed following injections placed into the ventral SC or into any subfield of the hippocampus. Injections of HRP were placed into the anterior thalamus in order to identify the cells of origin. HRP-positive cells were identified in a restricted zone within the SC and RS. These cells occupied the most superficial layer. The radioautographic data suggest a differential projection, along the medio-lateral axis of the dorsal SC and RS, to the anterior thalamus. Fibers from the lateral portion of the SC (adjacent to the hippocampus) project to the AM; fibers from the medial portion of SC (adjacent to RS) project to the ventral portion of the AV, and fibers from the RS project to the dorsal portion of the AV. While the SC projects through both the dorsal fornix and fimbria, other fibers could be traced along with fibers from the RS around the lateral ventricle and through the internal capsule to terminate in the anterior thalamus. In this way, it appears that the SC can modulate the activity of the anterior thalamus via two routes -- the fornix system and the internal capsule.

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560 THE RELEASE OF CELLULAR INHIBITION IN THE RAT HIPPOCAMPUS FOLLOWING MEDIAL SEPTAL LESIONS.

S. Walden Miller* and P. M. Groves. Dept. of Psych., Univ. of Utah, SLC, Utah 84112. Dept. of Psych., Univ. of Colo., Boulder, Colorado 80302.

The spontaneous and evoked activity of 142 neurons in the hippocampus of 44 rats were recorded, using extracellular microelectrodes. Activity was recorded both before and after lesions of the medial septal nuclei. Spontaneous patterns of activity varied appreciably between cells and could be classified according to a number of different criteria, the most obvious being, the presence or absence of spontaneous bursting. Approximately 1/3 of the neurons encountered displayed a regular, repetetive pattern, without bursts, and with spike frequencies commonly more than 12/sec. By contrast, the irregular, bursting pattern exhibited by the majority showed bursts of 2-7 spikes occuring from 1/5 to 12/sec.

The peripheral stimuli used to determine response properties consisted of light flash, auditory click and tactile stimulation. In the intact animal, 55% of the responsive neurons exhibited some period of inhibition. After medial septal lesions, only 21% of the neurons encountered showed inhibition. From a sample of 23 inhibited neurons, only 5 continued to show inhibition following medial septal lesions, while 13 displayed excitation only, and 5 showed no response. These findings suggest that the integrity of the medial septal nucleus is necessary for normal inhibition of many hippocampal units following peripheral stimulation.

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561 ROLE OF HIPPOCAMPUS IN SPATIAL DISCRIMINATION. David S. Olton, John A. Walker*, Fred H. Gage III*, and James Simmons*. Department of Psychology, The Johns Hopkins University, Baltimore, Md. 21218

The hippocampus has been suggested as important in the ability of animals to locate themselves in space. If such is the case, then destruction of the hippocampus itself or of areas closely related to the hippocampus ought to result in an impairment of spatial behavior. Rats were first trained on an eight arm radial maze in a paradigm which rewarded them for choosing each of the eight arms once without repeating choices. Analysis of data from normal animals indicated that they identified each of the eight arms by its position in space without relying on response chains, algorithms, or intramaze cues. Following training to criterion performance, radiofrequency lesions were made in the hippocampus proper, body of the fornix, entorhinal area, septum, postcommissural fornix, and neocortex. All lesions except those of the neocortex produced a severe deficit in performance, one which could be described as an active repetition of incorrect choices, often a repetition of a previous sequence of two, three, or even four choices. The data are discussed in terms of the functional connections of the hippocampus with other limbic structures and the types of deficits produced in spatial tasks following hippocampal lesions.

562 EFFECTS OF FORNICAL LESIONS ON CORTICOSTERONE LEVELS DURING EXTINCTION OF AN OPERANT RESPONSE. B. Osborne* and A.H. Black. Dept. Psychology, McMaster University, Hamilton, Ontario, Canada. L8S 4K1.

The purpose of this experiment was to study the effects of fornical lesions on corticosterone levels during the extinction of a simple lever pressing response in rats.

Two groups of rats (one with fornical lesions and one without) were trained to press a lever for food on a continuous reinforcement schedule. The lever pressing response was then extinguished. Corticosterone levels were taken throughout this procedure. In addition the animals' behaviour was video-taped.

Although the difference in resistance to extinction of pressing was small, the video-tape analysis revealed clear-cut differences between the behaviour of fornicals and controls. In addition, corticosterone level varied as the function of phase of training (less during acquisition than baseline and extinction). Finally, there was no difference in corticosterone levels between fornical and controls groups among animals housed in a general laboratory colony.

563 LIGHT AND ELECTRON MICROSCOPIC EVIDENCE FOR A PREFRONTAL-HIPPOCAMPAL PROJECTION IN MACAQUE. J. T. Povlishock,* G. R. Leichnetz and J. Astruc. Department of Anatomy, Medical College of Virginia, Richmond, Va. 23298.

Recently we have described a prefronal-hippocampal projection in the squirrel monkey (Brain, Behav., Evol. 11 (1975) 355-64). In order to ascertain whether a comparable connection existed in old world monkeys, we initiated this study in macaque by means of both light and electron microscopy. Ten rhesus (Macaca mulatta)and three cynomolgus (Macaca fascicularis) monkeys were subjected to unilateral partial ablations of the medial granular frontal cortex. After survival periods of 7-25 days, those monkeys used in the light microscopic study were processed with Nauta-Gygax and Fink-Heimer silver methods. The other monkeys, after survival periods of 48-72 hrs., were perfused with a paraformaldehyde-glutaraldehyde fixative and tissue samples from the ipsilateral hippocampus were removed and processed for electron microscopy. In the silver material prefrontal fiber degeneration was followed primarily through the cingulum, but also through the uncinate fascicle, to the hippocampal region. Fine caliber degeneration entered both the alvear and perforant paths. Degenerating fibers appeared to be given off the alvear path into the stratum oriens. The perforant path fibers traversed the stratum lacunosum-moleculare of CA1-3, and some degenerating fibers appeared to be given off into the stratum radiatum. The neuropil in the stratum pyramidalis contained some fine caliber debris suggestive of preterminal and terminal degeneration. At the electron microscopic level, degenerating axons, characterized by their electron density, have thus far been identified in the perforant path. Degenerating axon terminals demonstrating clustered synaptic vesicles and an electron-dense matrix were seen in synaptic contact with pyramidal cell dendritic profiles both within the strata lacunosum and radiatum. Most degenerating terminals contacted the dendritic surface proper and seldom the numerous dendritic spines so characteristic of these dendrites.

564 OLFACTORY EVOKED RESPONSES AND INDUCED SEIZURE ACTIVITY IN THE PRIMATE HIPPOCAMPAL FORMATION. Garl K. Rieke. Dept. of Anatomy, Hahnemann Medical College, Philadelphia, Pa. 19102.

Temporal lobe epilepsy involves seizure activity within the hippocampal formation. Temporal lobe seizures with an olfactory aura have been reported in some human epileptics. Current work on the squirrel monkey, a lower primate, has provided evidence that 1) the olfactory bulb transmits olfactory signals along axons of mitral cells directly to the hippocampal formation and 2) low frequency electrical stimulation of the olfactory bulb or lateral olfactory tract elicits a recruiting response recorded from the hippocampal and dentate gyri, and seizure patterns in EEG records from the temporal lobe. A biphasic response from the cortex of the parahippocampal gyrus had an onset latency of 4-6ms, could not be driven beyond 20-50Hz, and demonstrated little post-tetanic change. Long onset latency (40-50ms) responses were recorded from the dentate and hippocampal gyri. The infragranular plexus of the dentate gyrus is the probably generator of the dentate responses, since mirror image responses were obtained dorsal and ventral to this plexus. At low frequency (1Hz) stimulation of the tract or olfactory bulb, these responses increased in amplitude, and became biphasic. This configuration was maintained for up to 60sec and then decayed to the monophasic form. The duration of this response pattern ranged from 2.5-3min and is suggestive of recruitment. Concurrent EEG recordings showed spiking during the growth phase of the dentate or hippocampal responses. The spiking occurred in bursts suggestive of seizure like activity induced by olfactory stimulation. Recent observations in the rat, ferret and cat have shown a direct olfactory input to the cortex of the parahippocampal gyrus and the same conclusion can be drawn for the squirrel monkey. Further repetitive olfactory stimlation evoked seizure activity within the hippocampal formation.
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565 THE LIMBIC MIDBRAIN AS A RELAY BETWEEN LIMBIC CORTEX AND CEREBELLUM IN THE RAT AS DEMONSTRATED BY THE RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. J.A. Saint-Cyr and D.J. Woodward. Dept. Cell Biol., Univ. of Tex. Health Sci. Ctr., Southwestern Medical School at Dallas, Dallas, Texas, 75235.

The vermis and intermediate regions of the anterior lobe of the cerebellum were previously shown to be strongly activated by stimulation of the fornix (Saint-Cyr & Woodward, Neurosci. Abst., 833, 1975). This study was carried out to determine by which pathways this fornix activation of the cerebellum could be mediated. Injections (.15-.30µl) of horseradish peroxidase (HRP) were made into these cerebellar areas and revealed that rostral pontine nuclei as well as tegmental and reticular nuclei of the limbic midbrain area (LMA) were direct sources of fibers to these anterior cerebellar areas. Further injections (0.15-.30µl) of HRP were then made into the LMA to determine its sources of afferents. These sources included the bed nucleus of the stria terminalis, dorsomedial and posterior areas of the hypothalamus, the mammillary bodies, the supramammillary region and the adjacent zona incerta, the habenulae and the central grey. In addition, the cingulate (Rose's IRb and RSg) and adjacent supracallosal parasubicular cortical regions contained HRP-positive cells. These studies suggest several pathways via which fornix stimulation could influence the cerebellum. 1) The caudal supracallosal parasubicular cortex may relay monosynaptically via the fornix through the LMA while the RSg may do so via the cerebral peduncle. 2) Polysynaptic limbic projections via the fornix to the LMA may relay through the septo-habenulo-tegmental, bed nucleus of stria terminalis and hypothalamo-tegmental, or mammillotegmental routes. Thus, limbic cortical outflow may modulate cerebellar motor mechanisms as part of a system controlling spatial orientation.

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566 LEVER PRESSING OCCURS IN PHASE WITH HIPPOCAMPAL THETA RHYTHM IN RATS. Kazue Senba* and Barry R. Komisaruk. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

Based on the synchrony of vibrissa movements with theta rhythm in rats (Komisaruk,1970:JCPP,70,482) and hamsters (Macrides,1975:Behav.Biol.,14, 295), and sensorimotor modulation by alpha rhythm in humans (e.g. Lansing, 1957:Electroenceph.clin.Neurophysiol.,9,497; Harter & White,1967:Science, 156,406), we hypothesized that theta activity rhythmically modulates sensorimotor functions. The relationship between the timing of self-initiated lever pressing and the phase of theta cycle was studied in rats.

Stainless-steel parallel bipolar electrodes were implanted into the hippocampus. Nine rats were deprived of food in order to maintain 70-75 % of normal body weight, and learned that they could obtain one 50 mg food pellet each time they pressed a lever on an unrestricted schedule. When lever pressing was established, EEG and lever presses were recorded on a polygraph. Between 100 and 350 lever presses, which accompanied clear theta rhythm, were obtained in each of 9 rats in 1 or 2 sessions (a total of 1-5 hours). Each theta cycle was divided into 6 equal phases and the moment of each lever press was determined as a function of the phase.

Eight of 9 rats showed a significantly non-random distribution of lever presses in relation to the phase of the theta cycle. In 7 of these 8, there was a unimodal distribution of lever presses across the theta cycle. In 6 of these 7, the peak probability of lever pressing occurred during the negative phase of the theta rhythm, while one rat showed a peak in the positive phase.

These results indicate that the time of occurrence of a self-initiated motor act is modulated rhythmically in phase with the hippocampal theta rhythm.

567 ORIGINS OF THE AFFERENT CONNECTIONS OF THE MEDIODORSAL NUCLEUS IN THE RAT. Allan Siegel, Takeo Fukushima* and Henry Edinger. Depts. of Anatomy, Neuroscience, and Physiology, N.J. Med. School,Newark, N.J.07103.

The purpose of this investigation was to identify the cell bodies of origin of axons which supply the mediodorsal thalamic nucleus (MD). Injections of horseradish peroxidase (HRP) were placed into MD in 60 rats. Survival times varied from 1-4 days. Following injections placed into different portions of MD, HRP positively labeled cells were observed in a variety of structures which, in general, lie rostral to the injection sites. Discrete injections of HRP (.05µl) placed into the midline of MD labeled cells situated exclusively in posteroventral portions of the reticular thalamic nucleus. HRP injections limited to anterior levels of MD labeled cells in layers 4-6 of sulcal prefrontal cortex. Injections which involved more posterior portions of MD labeled cells in the same layers of the dorsomedial prefrontal cortex. Injections of this region of MD also labeled cells in neighboring regions of the anterior cingulate gyrus, olfactory tubercle, and sites immediately lateral to the vertical and horizontal limbs of the diagonal band. Labeled cells were observed in the cortical, medial, basolateral and basomedial amygdaloid nuclei and adjacent pyriform cortex only when ablations of the prefrontal cortex preceded HRP injections of MD. This observation suggests the possibility that axon terminals arising from cells in the prefrontal cortex prevent amygdaloid terminals from incorporating sufficient quantities of HRP required for successful labeling of its cells.

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568 MULTIPLE SOURCES OF INPUT TO PERICRUCIATE CORTEX OF THE CAT. Jerome Siegel and Henricus G.J.M. Kuypers. Dept. Anatomy, Erasmus Univ. Rotterdam, The Netherlands.

Somatic motor and sensory cortex (pericruciate region in the cat) receives its primary afferentation from the ventrobasal thalamus. Origins of direct pathways from the limbic system were investigated by retrograde transport of horseradish peroxidase (HRP) from pericruciate injections. HRP findings indicate that pericruciate cortex receives afferents from cells in the ventrobasal thalamus, claustrum, basal forebrain -diagonal band region, amygdala, and some cells in the midline and intralaminar thalamus, mesencephalic and pontine nuclei of the raphe, and locus coeruleus. The physiological characteristics of these connections were explored by recording changes in unit activity of precruciate cells to stimulation of ventralis lateralis (VL) of the thalamus and areas of the limbic system. The typical response of precruciate cells to VL stimulation was a short latency excitatory drive followed by inhibition. Stimulation to limbic structures resulted in longer and more variable latencies and often modulated the VL induced activity. Many units were driven into different firing patterns by stimulation to two or more limbic sites. A small proportion of cells was antidromically invaded by VL stimulation; few cells were antidromically driven by limbic stimulation. We conclude that many precruciate cells are monosynaptically driven by VL inputs and are additionally converged upon by afferents from two or more limbic areas. Limbic influences which were of stable latencies, regardless of length, were also presumed to be monosynaptically mediated. This study demonstrates the abundant possibilities which exist for limbic modulation of somatic processes and indicates that influences from more than one limbic structure converge and interact upon somatic elements. (Supported by NATO Research Grant No. 1018).

569 PROJECTIONS OF THE BASAL FOREBRAIN IN THE CAT. Raymond Troiano* and Allan Siegel. (Spon: S. Cook) Depts. of Neuroscience and Anatomy, N.J. Med. School, Newark, N.J. 07103.

The present study utilized the technique of ^{3}H-amino acid radioautography to identify the efferent connections of selected regions of the basal forebrain in the cat. Injections of ^{3}H-Leucine (0.2-0.3 µL., 20 µCi/µL) were placed into the nucleus accumbens, substantia innominata, olfactory tubercle, and septal area. Survival times varied from 1-2 days. The results indicate that fibers from the dorsal septum pass caudally in the basal forebrain and terminate in massive quantities in the preoptic area and anterior lateral hypothalamus. Axons arising from cells in the medial portion of the nucleus accumbens are distributed via the medial forebrain bundle to the lateral hypothalamus, ventral tegmental area, and medial part of the substantia nigra. Axons arising from cells in the lateral portion of the nucleus accumbens project through the medial forebrain bundle to more caudolateral levels of the midbrain and terminate in the region of the dorsal tegmentum and lateral part of the substantia nigra. In contrast, fibers from both the olfactory tubercle and substantia innominata project rostrally as well as caudally. Rostral projections include more anterior levels of the olfactory tubercle, medial septum, and the ventral aspect of the gyrus proreus. Caudal projections include the lateral preoptic area and posterior levels of the olfactory tubercle and substantia innominata.

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570 VISUAL AND OTHER SENSORY INPUTS TO THE AMYGDALA OF THE RHESUS MONKEY. Blair H. Turner, Mortimer Mishkin and Margaret E. Knapp*. Howard Univ., Washington, D. C. 20059 and NIMH, Bethesda, Md. 20014.

As part of an attempt to understand the sensory control of emotional processes we have examined sensory inputs to limbic structures. This report focuses on telencephalic sensory inputs to the amygdala, with special reference to vision. Animals with lesions of selected sensory neocortical areas or of the olfactory pathway were perfused after an 8-day survival period, and resulting degeneration was traced with the Fink-Heimer stain. The occipital projection to the temporal lobe was known from earlier studies to be limited to areas TEO and TE of Bonin and Bailey, areas shown by behavioral and physiological experiments to be exclusively visual in function. Destruction of TEO revealed heavy connections with TE, but not the amygdala. Ablation of TE, on the other hand, resulted in considerable degeneration in the anterodorsal part of the lateral and basolateral amygdaloid nuclei. Selective lesions of the ventral and dorsal parts of TE showed that the ventral part projects only to the lateral nucleus while the dorsal part projects mainly to the basolateral nucleus. A lesion of the anterior part of area TA, which anatomical and behavioral data have implicated in audition, led to degeneration in the lateral part of the lateral amygdaloid nucleus that overlapped the visual projection, but extended more ventrally and posteriorly than the visual. A lesion of the anterior parts of the frontal operculum and insula, which behavioral and physiological data have implicated in taste, resulted in degeneration within the central part of the basolateral and basomedial nuclei, and the dorsomedial part of the lateral nucleus. Finally, lesions of the olfactory bulb and tract led to degeneration in the medial part of the medial amygdaloid nucleus and layer IA of the prepiriform and periamygdaloid cortices. The results suggest that all sensory systems project to the amygdala but that their projection sites are relatively distinct.

571 BRAIN STEM SOURCES OF HIPPOCAMPAL THETA RHYTHM INVESTIGATED BY CRYOGENIC BLOCKADE OF HYPOTHALAMIC PATHWAYS IN THE CAT. Charles L. Wilson, Noriaki Hirasuna and Donald B. Lindsley. Depts. Psychol., Physiol., Psychiat., and Brain Research Institute, UCLA, Los Angeles, 90024.

Macadar, Chalupa and Lindsley (Exp. Neurol., 1974, 43, 499-514) have reported that stimulation of n. locus coeruleus, n. reticularis pontis oralis and ventrolateral periaqueductal gray substance elicits rhythmic slow wave activity (theta rhythm) in the hippocampus. In order to identify the ascending pathways by which these brain stem structures influence hippocampal electrical activity cryoprobes were placed bilaterally in medial and lateral hypothalamus for producing reversible cryogenic blockade. Hippocampal response to stimulation of these brain stem regions was determined before, during and after cooling of hypothalamic regions to 5°C.

Operative procedures were carried out under halothane anesthesia and recordings were obtained during artificial respiration on a 1:1 mixture of N_2O and O_2 after immobilization with gallamine triethiodide and infiltration of wound margins and pressure points with 1% lidocaine at regular intervals. Respiratory CO_2 level was maintained at 4% and rectal temperature at 36 to 38°C. Electrical stimulation thresholds for inducing hippocampal theta rhythm were determined for each of the brain stem sites using 100 Hz stimulation trains of 5-8 secs duration.

Spontaneous hippocampal theta rhythm was decreased or blocked by hypothalamic cooling and replaced by irregular slow wave activity; neocortical activity manifested prominent desynchronication or activation during cooling. Hypothalamic cooling either blocked or markedly attenuated hippocampal theta rhythm induced by brain stem stimulation; differential effects depending upon sites of cooling and stimulation will be discussed.
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572 ELECTROPHYSIOLOGICAL CORRELATES OF BEHAVIOR MEASURED IN THE HIPPOCAMPAL FORMATION OF THE FREELY MOVING RAT. Jonathan Winson, The Rockefeller University, New York, NY 10021 and Charles Abzug, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

Experiments were designed to answer the following question: Are different behavioral conditions accompanied by alterations in the pattern of excitatory and/or inhibitory synaptic activity impinging upon neurons in the various architectonic subdivisions of the hippocampal formation? To study this problem, various chronic devices for stimulation and recording were implanted in rats. The recording apparatus included one facility for advancement of a microelectrode through the hippocampal formation on one side and several fixed macroelectrodes on the contralateral side. In addition, stimulating electrodes were implanted in various locations in different animals. After an animal had recovered from anesthesia it was allowed to roam freely within a small enclosure while both electrical stimulation and recording of activity were carried out. The behavioral state of the animal was determined by a combination of visual observations of its activity and study of the simultaneous record of hippocampal electrical activity as recorded at the fixed macroelectrodes. Electrical stimuli were applied at a series of intensities during the occurrence of slow wave sleep (SWS), rapid eye movement sleep (REM), and during two conditions of alertness, one in which the animal was stationary (quiet alert, QA), and the other in which the animal was engaged in voluntary motion (VM). Our results establish that during the QA state, inhibition is exerted tonically upon the granule cells of the dentate gyrus, while during SWS such inhibition is either not present at all or is diminished in magnitude. During REM and VM, there are in addition further synaptic effects exerted upon pyramidal cells in the CA1 and CA3 regions.
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Membrane Structure and Function

573 THE EFFECT OF TEMPERATURE ON CALCIUM TRANSPORT IN MYELINATED NERVE OF FROG. F.P.J. Diecke, Marguerite A. Stout and Stanley Greenberg. Dept. of Physiol., CMDNJ-New Jersey Medical School, Newark, N.J. 07103 and Dept. of Pharmacol., Ohio State Univ., Col. of Med., Columbus, Ohio 43210.

The temperature dependence of calcium transport in squid axon has been reported to have a Q_{10} of 2.54 (Luxoro and Yanez, 1968) and 2.3 (Blaustein and Hodgkin, 1969), corresponding to energies of activation of 15,300 and 13,500 cal/mole respectively. In contrast to these high values for activation energy, a considerably lower temperature dependence of calcium transport has been observed in vertebrate tissues. Q_{10} values of 1.41 for cardiac muscle (Reuter and Seitz, 1968) and 1.4 for rabbit vagus nerve (Kalix, 1971), corresponding to energies of activation of 5,900 and 5,640 cal/mole respectively, have been reported.

We have investigated the dependence of calcium influx and efflux in desheathed bundles of myelinated nerve of frog. In normal Ringer solution, over a temperature range from 7°C to 37°C, calcium efflux changed with a Q_{10} of 1.47 indicating an energy of activation of 6,500 cal/mole. A Q_{10} of 1.4, corresponding to an activation energy of 5,600 cal/mole, was measured for calcium influx. These values are in close agreement with those observed in vertebrate cardiac and smooth muscle and rabbit vagus nerve.

Since vertebrate tissues represent multicompartmental systems, the measured rates of calcium influx and efflux represent a complex function of the rates of membrane transport, extracellular diffusion and the exchange with intracellular sequestering sites. As a consequence, the observed Q_{10} would be a function of the temperature dependence of all compartments. These potential errors can be avoided if the transmembrane flux of calcium is greatly reduced relative to the rates of extracellular diffusion and intracellular exchange, so that the transmembrane flux becomes the truly rate limiting step. Calcium transport can be effectively reduced with extracellular lanthanum (vanBreemen and DeWeer, 1970). We have therefore, measured calcium transport as a function of temperature in Ringer solutions containing 5 mM lanthanum. In the presence of lanthanum ions, calcium influx and efflux are reduced to approximately 7% of that in Ringer solution without a change in extracellular diffusion. The values for Q_{10} and activation energy, are in close agreement with those observed in normal Ringer solution (Q_{10} for influx = 1.46, Q_{10} for efflux = 1.43). The data indicate that the temperature dependence of calcium transport in vertebrate excitable tissues differs significantly from that observed in squid giant axons and suggests that the mechanism regulating the intracellular calcium concentration may differ also. (Supported by grant NS-05188 from NINCDS.)

574 STRUCTURAL DIFFERENCES OF MEMBRANES IN THE PACINIAN CORPUSCLE: A FREEZE-FRACTURE STUDY. Robert B. Hanna*, Peter S. Spencer*, George D. Pappas. Depts. of Neuroscience and Pathology, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, The Bronx, New York 10461.

The pacinian corpuscle is a specialized mechanoreceptor responsive to vibration stimuli. The principal component of the pacinian corpuscle is an outer core formed of cellular lamellae separated by fluid-filled interlamellar spaces. The outer core, which is continuous with the perineurium, is penetrated proximally by a central canal and a capillary network. Passing through the canal is a single, myelinated, preterminal nerve fiber 7-11 μm in diameter. This is continuous with a long, non-myelinated nerve terminal lying deep in the center of the corpuscle, and surrounded by compacted inner core cell processes (hemilamellae) and interlamellar collagen fibers. The terminal axon is elliptical in cross-section with long, filopod processes emanating from the poles. The tips of axon processes are intimately apposed to the free sides of the two stacks of hemilamellae which together comprise the inner core. At the distal extremity of the inner core, the axon may branch or form a bulbous ultraterminal region which exhibits numerous filopod axon processes emanating from the entire surface in a hydra-like array. The axolemma of the non-myelinated axon is believed to be the site of mechano-electrical transduction.

The technique of freeze-fracture was utilized to examine the membranes comprising pacinian corpuscles in the mesocolon of five cats. The membranes of the outer core exhibit a loose meshwork of ridges, tight junctions, and numerous pinocytotic pores 40 nm in diameter. These characteristics are similar to those reported for membranes of the perineurial sheath of the sciatic nerve (Reale et al., 1975, J. Neurocytol. 4:261-70). The membranes of the inner core hemilamellae are, however, quite distinctive. The P face shows a large number of particles (1600/μm^2). These particles are 10 nm in diameter and are uniformly distributed over the entire membrane. In addition, small gap junctions ~150 nm wide are found between adjacent hemilamellae. Pinocytotic pores are fewer in number and larger in size when compared with those in the outer core. The axonal membrane (P face) contains an extremely large number of particles (3000/μm^2). The majority of these particles measure 6.5 nm in width, and a smaller proportion 10 nm in width. The filopod processes also exhibit this particle composition. The E face of the axonal membrane is relatively smooth and aparticulate. The particle density observed in this specialized receptor membrane is much greater than seen in other non-myelinated axonal membranes.

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575 BINDING OF ANTI-ACETYLCHOLINE RECEPTOR ANTIBODIES TO RECEPTOR -RICH MEMBRANES FROM TORPEDO ELECTRIC TISSUE VISUALIZED WITH FERRITIN CONJUGATES. Arthur Karlin*, Eric Holtzman, Ramon Valderrama*, Konrad Hsu*, and Fe Reyes*. Depts. Neurol., Biolog. Sci., and Microbiol., Columbia University, N.Y., N.Y. 10032.

Antisera to purified acetylcholine receptor were raised in rabbits (Valderrama, et al., Ann. N.Y. Acad. Sci., 274: 108, 1976). The binding of antibodies in these sera to receptor -rich membranes was determined by both the direct and indirect immunoferritin methods. In the direct method, IgG isolated from immune or preimmune sera was conjugated to ferritin with m-xylylene diisocyanate. The ferritin-conjugated IgG was incubated with plasma membrane vesicles from Torpedo californica electric tissue (Hazelbauer and Changeux, Proc. Nat. Acad. Sci., 71: 1479, 1974). Receptor comprises 10 to 20 per cent of the protein of these vesicles. The treated vesicles were trapped and washed by filtration on Millipore filters (25 to 50 µg of membrane per filter). In the indirect method, vesicles were incubated with heat-treated sera, trapped and washed on Millipore filters, incubated on the filters with ferritin-conjugated sheep anti-rabbit IgG, and washed again. In both methods, vesicles on the filters were fixed in glutaraldehyde and dehydrated in ethanol. The filters were dissolved in propylene oxide. A thin film of cross-linked protein and lipid is left which was embedded in Epon for electron microscopy (Baudhuin, et al., J. Cell Biol., 32: 181, 1967).

With both the direct and the indirect methods, there is extensive binding of antibody to the outside surface of the vesicle membrane. When the membrane is stained to bring out the usual trilaminar structure, the electron dense core of the attached ferritin is located on the order of 10 nm from the surface of this structure. Although this separation is undoubtedly partially due to the geometry of the ferritin-antibody conjugate, it may also reflect a protrusion of the pertinent antigenic sites of the receptor from the membrane surface. When preimmune sera are used in the indirect method or ferritin conjugated to normal IgG in the direct method, there is little attachment of ferritin to the vesicle membrane. This is also the case when anti-receptor sera are preincubated with excess purified receptor before addition to vesicles. Furthermore, there is little binding of the antisera or IgG's to membranes of a total particulate fraction of Torpedo liver. Thus, the methods described here for the electron microscopic localization of acetylcholine receptors in membranes appear to be sensitive and specific and should prove useful for the detailed localization of receptor antigenic sites.

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Figs. a and b. Anti -receptor binding visualized by the indirect method. In b, anti-receptor serum was incubated with excess purified receptor and then vesicles.

576 GLYCOPROTEINS OF ISOLATED SYNAPTIC JUNCTIONS. Paul T. Kelly and Carl W. Cotman. Dept. Psychobiol., Sch. Biol. Sci., UCI, Irvine, CA 92717

Glycoproteins have been shown to play an important role in intercellular recognition and adhesion in a variety of non-neural systems. By analogy, specific brain glycoproteins may have an equally critical function in the formation and maintenance of synaptic junctions (SJ). We have begun to examine certain classes of glycoproteins in isolated SJs following electrophoresis in the presence of SDS on polyacrylamide slab-gels. Proteins and glycoproteins have been identified by Coomassie Blue (CB)-staining and characterized according to apparent molecular weights and per cent composition. Specific glycoproteins containing galactose and/or galactoseamine were labeled by oxidation with galactose oxidase followed by reduction with tritiated NaB^3H_4. Although nearly all SJ components displayed some galactose oxidase-dependent labeling, high levels of incorporation were localized to four molecular weight regions (110-145 K, 70-75 K, 35-40 K and 13-25 K daltons).

In order to elucidate the role of synaptic glycoproteins in neural recognition and adhesion it is necessary to identify those components which are located on the external membrane surface and extend into the synaptic cleft. We have previously shown using ferritin-Concanavalin A (Con A) conjugates that nearly all Con A binding sites present in isolated SJs are localized to the outer surface of the postsynaptic membrane. The molecular counterparts to the Con A binding receptors of intact SJs have been identified by examining the binding of I^{125}-Con A to gels following the electrophoretic separation of detergent solubilized SJs. Four major Con A binding glycoproteins (apparent M.W. 160-165 K, 123 K, 108-118 K and 95-100 K daltons) are found greatly enriched in SJs. Two of these glycoproteins represent minor components as determined by CB-staining.

In summary, these results identify two classes of SJ glycoproteins: one class containing galactose and/or galactoseamine and the other containing Con A binding saccharides. Interestingly, the three major CB-staining components of SJs do not contain significant amounts of the carbohydrates resolved by these methods. Most important, however, these data provide the first identification of a specific class of glycoproteins located on the external surface of the postsynaptic membrane of CNS synapses.
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577 A RAPID METHOD FOR THE IDENTIFICATION OF SYNAPTIC MEMBRANE LECTIN BINDING GLYCOPROTEINS IN POLYACRYLAMIDE GELS. John A.P. Rostas*, Carl W. Cotman and Paul T. Kelly (SPON: J.V. Nadler). Dept. Psychobiol., Sch. Biol. Sci., UCI, Irvine, CA. 92717.

Plant lectins display specific affinities for carbohydrate residues and are proven analytical tools in the study of specific glycoprotein components of cell membranes. Using these ligands we have developed a rapid method for studying the nature and distribution of glycosylated components in synaptic plasma membrane (SPM) fractions and in synaptic junctional complex (SJ) and postsynaptic density (PSD) fractions, which are prepared from SPM fractions by treating them with Triton X-100 or N-lauryl sarcosinate, respectively. The glycoproteins of these synaptic fractions are separated on a high resolution SDS polyacrylamide gel electrophoresis system and then, without chemical modification, the individual lectin binding components can be identified by directly applying I^{125}-labelled lectins to the gel. The bound lectin can be localized by autoradiography and then quantified by counting gel slices in a scintillation counter.

Both detergent treatments were found to solubilize from SPM a group of Concanavalin A (Con A) binding glycoproteins with apparent molecular weights between 45 K and 52 K daltons. Four high molecular weight Con A binding glycoproteins (160-165 K, 123 K, 108-118 K and 95-100 K daltons) were identified in the SJ fraction. Only the 160-165 K and 108-118 K dalton glycoproteins were present in the PSD fraction. Most of the bound Con A in this fraction was associated with components that migrated with the dye front, suggesting that they may be glycolipids. The results obtained with additional lectins specific for different carbohydrate residues will also be presented. The only method previously available for the identification of glycoproteins resolved on polyacrylamide gels takes several days and involves chemical cross-linking of the glycoproteins. Our data therefore suggest that plant lectins may be useful in the localization and identification of synaptic glycoproteins in brain.
(Supported by NIH Research Grant NS 08597)

578 A MICROSCOPIC THEORY OF THE THRESHOLD POTENTIAL IN NERVE EXCITATION. SHANG J. YAO, SIDNEY K. WOLFSON, JR. Dept. Neurol. Surg., Univ. of Pittsburgh, and Surg. Res. Lab., Montefiore Hosp., Pittsburgh, PA. 15213 and HUEI-YING SUN YAO*, 1695 Hastings Mill Rd., Pittsburgh, PA. 15241.

Much attention has been paid to the understanding of the action potential. However, little is known about the molecular origin of the threshold. In principle, the threshold potential plays a crucial role in nerve excitation. It has been known for many years that there exists a nonlinear relation between the applied potential and ion permeability in steady state voltage/current characteristics (V/I) of nerve membranes. The physical implication of this nonlinearity was unclear. When plotted into two different scales of current (I), it reveals[1] that in the low V region, V/I is linear in the linear I-scale graph: whereas in the high V region, V/I is linear in the log I plot, i.e., V is plotted against log I. The switching of the V/I in the two plots should imply a switch in molecular mechanism during excitation. This switching, occurring in almost all types of biological membranes and at around 40 mV displacement from their resting potentials, was recently recognized and interpreted as the threshold phenomenon.[2]

This paper will present a more detailed discussion on molecular processes occurring around the threshold, specific to the nerve excitation. From the V/I point of view, in the low V region, the observed ionic current should be Ohmic. The I should be due mainly to the migration of ions in response to the applied V and thus V/I is linear. No electrochemical reaction should ever occur. This region is considered here as the subthreshold. In the high V region, the ionic current varies with the exponential of the applied V, i.e., $I \propto \exp(AV)$ where A is a constant. This is in accordance with the Tafel plot or the simplified Butler-Volmer equation. Such a phenomenon is related to electron-transfer at an electrode/electrolyte interface. In the present case, it implies that the rate determining step is the electron-transfer at one side of the nerve membrane/electrolyte boundary. This interfacial electron-transfer occurring at body temperature should be mainly attributed to electron tunneling across membrane/electrolyte interface. The observed ionic current is manifested as a consequence of the electron-transfer process. The voltage at which the molecular mechanism switches during excitation is the voltage when the observed current changes from Ohmic to Tafel-like. It is also the voltage at which the applied potential reaches the threshold. At the threshold, the Fermi-level of the semiconducting nerve membrane matches[3] that of the redox electrolyte and permits the tunneling of electron across interface.

It becomes clear that subthreshold is associated with simple ionic migration whereas suprathreshold is involved with an interfacial electron-transfer process.

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579 DISTRIBUTION OF SPECIFIC (^{3}H) OUABAIN BINDING TO NEURONAL AND NON-NEURONAL MEMBRANE FRACTIONS OF SKELETAL MUSCLE. Shailesh P. Banerjee, Virendra K. Sharma* and Satya R. Dasgupta* (Spon. Eleanor H. Boyd). Dept. of Pharmacol. and Ctr. Brain Res., Univ. of Rocester, Roch. N. Y. 14642.

Since (^{3}H) ouabain (OU) specifically binds to ($Na^+ + K^+$)-ATPase of cell plasma membrane, an estimate of the distribution of ($Na^+ + K^+$)-ATPase on the neuronal and non-neuronal membrane fractions of rabbit skeletal muscle was obtained by measuring specific (^{3}H) OU binding to muscle microsomal fractions before and after 6-hydroxy dopamine (6-OH DA) treatment in normal and sciatic nerve denervated skeletal muscles. The specific (^{3}H) OU binding to the microsomal fractions obtained from innervated anterior tibial, gastrocnemius and soleus muscles were 1.5, 5.2 and 9.2 p moles/mg protein respectively. In 14 days sciatic nerve denervated anterior tibial, gastrocnemius and soleus muscles these values were 1.6, 7.0 and 10.1 p moles/mg protein. Two weeks after 6-OH DA treatment, the specific (^{3}H) OU binding decreased to 1.1, 1.3 and 1.1 p moles/mg protein in sciatic nerve innervated anterior tibial, gastrocnemius and soleus muscles respectively while in 14 days sciatic nerve denervated muscles these values were found to be 1.2, 2.2 and 1.4 p moles/mg protein. These results indicate that specific (^{3}H) OU binding in red, slow muscle is greater than in pale, fast muscle. Again, the major fraction of specific (^{3}H) OU binding appears to be localized in the sympathetic nerve endings. Finally, there is some tendency of increased specific (^{3}H) OU binding in sciatic nerve denervated muscle microsomal fraction with or without 6-OH DA treatment. (Supported in part by the American Heart Association and the N.I.H. grant, HL-18185-01.)

580 LIPID COMPOSITION OF BOVINE CNS AXOLEMMA. George H. De Vries and Richard G. Saul*. Dept. of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virgnia 23298 U.S.A.

Bovine CNS axolemma-enriched fractions (AXL) and myelin were simultaneously prepared from purified myelinated axons as previously described (Exp. Brain Res. 23, 236, 1975). AXL was 50% lipid on a dry weight basis. On a dry weight basis the AXL was 27% cholesterol, 23% galactolipid (cerebroside and sulfatide in a molar ratio of 4 to 1) and 49% phospholipid comprised of 5.9% sphingomyelin, 15.8% choline phosphatides, 11.3% ethanolamine phosphatides, 5.7% phosphatidyl serine and 5.8% phosphatidyl inositol. Gangliosides comprised about 1% of the lipid weight. The AXL fraction was relatively free of contamination by endoplasmic reticulum as judged by NADPH-cytochrome c reductase activity and mitochondrial contamination as indicated by both cytochrome oxidase activity and cardiolipin levels. Relative to myelin the AXL fraction was enriched in phospholipid (particularly choline phosphatides) and gangliosides but contained a similar level of galactolipid. On the basis of this lipid profile as well as the previously determined enzymatic profile we conclude that the galactolipid in the AXL cannot be entirely due to myelin contamination but is present as an intrinsic membrane component.

(Supported by NIH Grant NS10821-03)

581 THE DISTRIBUTION OF MEMBRANE MOLECULAR SPECIALIZATIONS CHARACTERISTIC OF THE NODE OF RANVIER IS NOT DEPENDENT UPON MYELINATION. Mark H. Ellisman. Dept. Mol. Cell. and Dev. Biol., U. of Colo., Boulder, Colorado 80302.

Strain 129/ReJ-Dy "dystrophic" mice have a genetic deficit in Schwann cell development and myelinogenesis (Stirling, J. Anat. 119: 169, 1974). Ventral roots of these animals are characterized by regions with partial or incompletely myelinated axons as well as large axons entirely lacking in Schwann cell wrapping. The myelination and nodal specializations appear normal in nerve axons peripheral to the spinal roots of these same animals. Utilizing quantitative transmission electron microscopy of freeze fracture replicas, the nodal, paranodal, and interparanodal axolemmas are differentiable according to particle size and density. Protoplasmic fracture faces of normal nodes of Ranvier contain 100Å particles at a density of 400/um^2 and about 250 elongated 150-250Å particles/um^2. The particles of non-nodal regions of membrane (paranodal and interparanodal) are of smaller diameters and different densities. Therefore the nodes of Ranvier may be recognized by their characteristic number, size and distribution of particles. Dystrophic mice peroneal nerve axons appear normally myelinated and have approximately the same distributions of particles as normal axons. However, in some regions of the dystrophic ventral roots, Schwann cell wrappings may be missing from entire internodal regions, creating "hemi nodes" of Ranvier where myelination terminates. At the hemi node there is a band of axonal membrane with a particle size density distribution similar to that found in normal nodes. In the amyelinated portions of the ventral roots many large axons lie adjacent to one another and their membranes exhibit patches of particles that have particle distributions characteristic of nodal membrane. Thus, the nodal membrane has a characteristic particle profile that is not necessarily dependent upon local restriction by Schwann cells. (Supported by a grant from M.D.A.A. to Keith R. Porter.)

582 CYTOCHEMICAL LOCALIZATION OF 2',3'-CYCLIC NUCLEOTIDE 3'-PHOSPHOHYDROLASE IN CNS WHITE MATTER. Edgar L. Engel and John G. Wood. Dept. Anat., UTCHS, Memphis, TN. 38163.

The enzyme 2',3'-cyclic nucleotide 3'-phosphohydrolase is a membrane-bound enzyme known to be especially active in CNS myelin fractions (Drummond et al., J. Biol. Chem. 237: 3535, 1962; Kurihara et al., J. Neurochem. 14: 1167, 1967). In an effort to determine the precise distribution of this enzyme in myelin and its associated oligodendrocyte membranes, a cytochemical technique was utilized to localize the enzyme. Rabbit corpus callosum fixed by perfusion with 4% paraformaldehyde, .1% glutaraldehyde and .25M dextrose in .2M cacodylate buffer(pH 7.4) was sliced into 50μ sections. These tissue slices were preincubated for 45 min. in 150 μg/ml alkaline phosphatase in .1M Tris-maleate buffer(pH 7.4) containing .25M sucrose at 30°C, followed by incubation for 30 min. in the same buffer containing 150 μg/ml alkaline phosphatase, 3mM 2',3'-cAMP and 2mM lead nitrate. The slices were then osmicated and embedded for electron microscopy. Unstained thin sections revealed electron-opaque product in association with the glial membrane adjacent to the axolemma and with the internal mesaxon. Product was seen along the internal mesaxon but did not continue into the compact myelin lamellae. Opaque deposits were occasionally associated with the outside of the myelin sheath. In addition to the observations on myelin membrane, red blood cell membranes also showed activity. In both cases, product was localized on the outside of the membrane. Several control experiments demonstrated the validity of this technique. The possibility exists that this enzyme is sequestered in certain regions of the myelin membrane. (Supported by USPHS Grants 5T01-GM00202 and NS-12590. JGW is a Sloan Fellow.)

583 BEHAVIORAL AND ELECTROPHYSIOLOGICAL CHANGES IN MICE FOLLOWING TRIETHYL TIN TREATMENTS. R. A. Gerren*, D. E. Groswald* and M. W. Luttges. Dept. Aero. Eng. Sci., University of Colorado, Boulder, Colorado 80309.

Spontaneous and elicited behavioral tests were coupled with electrophysiological measures to evaluate the effects of triethyl tin on mice. These measurements were completed to assess chronic triethyl tin toxicity as a model for demyelinative disorders. Spontaneous locomotor activity was significantly lower in mice which received chronic daily injections of triethyl tin (2 mg/Kg body weight) than in mice which received control injections of saline. Upon 30 successive days of testing the triethyl tin mice exhibited significantly lower levels of spontaneous activity with continued daily treatments. On the final test day mice received an additional triethyltin injection and were then allowed to rest for two days before active avoidance testing. They performed as well as untreated control mice when tested in an active avoidance task. Sciatic nerve excitation thresholds and conduction velocities were found to be increased in triethyltin treated mice. This was true whether mice received single (8 mg/Kg body weight) or repetitive daily triethyltin injections prior to nerve recording. Cortically implanted mice received single triethyltin injections prior to electroencephalographic (EEG) recording. Frequency analysis of the EEG records show that the triethyltin mice exhibit lower frequency activity than do control mice. The alterations persist for approximately two hours after a single injection of triethyltin but are not evident twenty-four hours later. These behavioral and electrophysiological alterations induced in mice by triethyltin suggest that triethyltin may have limited usefulness as a model for demyelinative disorders of the nervous system.

584 BARBITURATES BLOCK Ca SPIKES BUT NOT Na SPIKES IN APLYSIA NEURONS. James M. Goldring*and Mordecai P. Blaustein. (SPON: R.A.Ratcheson) Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO, 63110.

The effects of low concentrations of pentobarbital on the action potential of the identified cell, R_2, of the Aplysia abdominal ganglion have been studied. In this cell, during the rising phase of the action potential, both Na and Ca carry inward current, through pharmacologically distinct channels, and pure Na or Ca spikes can be generated (J.Physiol. 199:347,1968). Standard electrophysiological techniques were used to stimulate and record from the cell; the maximum rate of rise $[(dV/dt)_{max}]$ of the spike was also measured. Low concentrations of pentobarbital ($\leq$0.5 mM) had no effect on resting potential or input resistance of the cell. In Na-free sea water, with a normal $[Ca]_o$ of 11 mM, $(dV/dt)_{max}$ of the Ca spike was greatly reduced by 0.05-0.2 mM pentobarbital. With $[Ca]_o$ raised to 30 mM, 0.1-0.5 mM pentobarbital caused a reversible, dose-dependent decrease in $(dV/dt)_{max}$ of the Ca spike, with 0.4 mM causing about a 50% reduction. These concentrations of pentobarbital had no discernible effect on the Na spike measured in Ca-free sea water. A pentobarbital concentration of 1 mM was required to decrease $(dV/dt)_{max}$ of the Na spike 10%. We conclude that low concentrations of pentobarbital may selectively inhibit certain Ca conductance systems. This is of interest because, in some cases (e.g., Brit.J.Pharm.22:415,1964), barbiturates have been found to depress synaptic transmission by reducing the amount of transmitter released. One explanation (Mol.Pharm.11:369,1975) is that barbiturates may block increases in Ca conductance at the pre-synaptic terminal, as they do in R_2, thereby reducing Ca entry and transmitter release.

585 PROTEIN LOCALIZATION IN PNS MYELIN USING LACTOPEROXIDASE IODINATION. Ronald W. Gruener*and Richard G. Peterson, Dept.of Neurobiol. and Anatomy, The Univ. of Texas Med. Sch. at Houston, Houston, TX 77025.

Lactoperoxidase iodination has been previously used to study the localization of proteins in various membrane systems, including red blood cells (Phillips and Morrison, Biochemistry, 10:1766, 1971) and CNS myelin (Poduslo and Braun, J. Biol. Chem. 250:1099, 1975). In this study, lactoperoxidase iodination was used to study the localization of proteins in PNS myelin. Both prepared myelin and whole split sciatic nerves from mice were iodinated using the method of Hogg (Proc. Nat. Acad. Sci. 71:489, 1974). Myelin was prepared from the nerves which were iodinated and gels (of prepared myelin samples) were run (Fairbanks et al, Biochemistry, 10:2606, 1971). After staining and scanning, the gels were sliced into 1mm segments and the ^{125}I in each slice was counted. Comparison of gel scans and iodine labeling indicated the two glycoproteins P_o and X were labeled while the basic proteins, P_1 and P_2 were either not labeled or were labeled to a much lesser extent, depending on the amount of disruption which had taken place in the myelin. These results indicate that the P_o and X proteins are exposed in the intraperiod band or outer surface of the myelin membrane, while the basic proteins are localized in the main period band or inner surface.

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586 GAP JUNCTIONAL COMMUNICATION DURING DIFFERENTIATION OF NORMAL AND FUSION-ARRESTED CHICK MUSCLE CELLS IN CULTURE. Nurit Kalderon*, Miles L. Epstein and Norton B. Gilula*. The Rockefeller University, New York, N.Y. 10021.

Myogenesis has been studied in primary cultures of myoblasts from 11-day chick embryonic thigh muscle to determine: (1) if gap junctional communication exists between myoblasts; and (2) the potential role of junctional communication in regulating myoblast fusion. Junctional communication was analyzed in both normal and fusion-arrested cultures with ultrastructural (thin-section and freeze-fracturing) and electrophysiological (low-resistance coupling) techniques. Samples from 30-36 hour cultures were fixed and examined ultrastructurally in the regions where myoblasts were closely apposed, but not yet fused. Typical gap junctions and low-resistance pathways were found between apposed myoblasts. The gap junctions range in size from 20nm to 300nm in length, there are 3-5 junctions between cells, and they exist as small plaques or linear strands (with 10-60 particles) on the P fracture faces. In order to clarify the role of gap junctional communication in the fusion process, similar studies were made on fusion-arrested cell cultures. Fusion-blocked myoblasts were obtained by treatment with either 1.8mM of the Ca^{++} chelator, EGTA, or with a low concentration (3.3×10^{-6}M) of the thymidine analogue, 5-bromodeoxyuridine. In both cases, the treatments appear to block fusion selectively without any apparent inhibition of the major events that mark the course of muscle differentiation. Gap junctions are present between the fusion-arrested cells with both treatments. In conclusion, gap junctions definitely exist between myoblasts prior to fusion; however, while junctional communication may be an essential interaction during myogenesis, it is not sufficient to generate myoblast fusion. (N. Kalderon is a Muscular Dystrophy Association of America, Postdoctoral Fellow. Supported by N.I.H. Grant # HL 16507).

587 PITUITARY SODIUM-POTASSIUM ADENOSINE TRIPHOSHATASE, (ATPase) ACTIVITY: MODIFYING EFFECT OF ESTRADIOL-BENZOATE (EB) AND EB + PROGESTERONE (P). James F. Knudsen, Department of Physiology, School of Medicine, University of Maryland, Baltimore, Maryland 21218.

Both electrolyte and organic substrate transport across the plasmalemma can be coupled to ATP hydrolysis; a process catalyzed by ATPase. The ability of EB (10µg/100gBW, administered sc or 1µg/100gBW/ 3 days) and P (5 mg/100gBW administered in conjunction with EB) to influence post-castrational (OVX) elevations in plasma gonadotropins (FSH and LH) and pituitary ATPase were scrutinized. Pituitary ATPase activity (defined as the difference in hydrolysis between reactions containing ouabain and those without ouabain) from OVX rats previously treated with EB (10µg) and sacrificed 1 hr subsequent to treatment increased 69% over OVX controls. Plasma LH and FSH were significantly ($p<0.01$) suppressed over controls. Twelve hrs following EB treatment, ATPase activity was not different from controls. Similarly, plasma LH at this time period was not significantly different than controls. However, plasma FSH was significantly suppressed ($p<0.05$) at both the 1 and 12 hr time periods after EB treatment. A chronic 3 day EB treatment (1µg) elicited an analogous response to that evidenced following the acutely administered EB; LH and FSH were decreased and pituitary ATPase was elevated. P administered in conjunction with EB evoked a 70% rise in the pituitary ATPase activity over control levels within 1 hr of treatment. Plasma LH levels were decreased to intact control values (10ng/ml). Contrary to the ability of EB to suppress plasma FSH when administered alone, EB administered with P failed to alter the plasma FSH levels, relative to controls. From these data, it is interesting to speculate that EB or EB + P treatment by increasing pituitary ATPase activity not only regulates the flux of metabolites into cells, and therefore possibly the synthesis and/or degradation of cellular components but also the responsiveness of the pituitary gonadotropic cell to stimuli.

588 CONDUCTANCE CHANGES PRODUCED BY ACETYLCHOLINE IN EEL ELECTROPLAQUE. Nancy L. Lassignal* and A.R. Martin. Dept. Physiol., Univ. Colorado Medical Center, Denver, CO 80220.

Single eel electroplaque cells were mounted horizontally between two chambers. The innervated face of the cell was exposed to the upper chamber through a small hole and was accessible for measurement of membrane potential and for iontophoretic application of acetylcholine (ACh) from a micropipette. The membrane potential could be altered by passing current between the two chambers. Tetrodotoxin (0.5 ug/ml) was added to the upper solution to prevent initiation of action potentials. At the normal resting potential (-90 mV) application of ACh produced a depolarization (the "ACh potential") which decreased as the membrane potential was reduced, reached zero at about zero membrane potential and reversed polarity at more positive membrane potentials. In the absence of divalent cations, reducing external Na from 160 mM to 32 mM by substituting glucosamine caused the reversal potential to shift about 12 mV in the negative direction. Increasing external K from 2.5 mM to 32 mM had little effect on the reversal potential in the presence of 130 mM Na. However in the glucosamine solution with 32 mM Na, the same increase in K concentration resulted in a 12 mM shift of the reversal potential in the positive direction. Reducing extracellular Cl from about 160 mM to 2.5 mM by replacement with isoproprionate had no detectable effect on the reversal potential. In Na-free glucosamine solution with 32 mM K, addition of 20 mM Ca changed the reversal potential from about -25 mV to about -13 mV. These results indicate that ACh increases the conductance of the electroplaque membrane to Na, K, and Ca, but not to Cl.

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589 FLOURESCEIN DYES EFFECT MEMBRANE PERMEABILITY OF MOLLUSCAN NEURONS. Herbert Levitan. Dept. of Zoology, Univ. of Maryland, College Park, Md. 20742.

Several dyes of the xanthene type (derivatives of flourescein) are added to foods and drugs in order to make their physical appearance more attractive to the human consumer. Inspite of the precautions taken to insure that such non-nutritive additives have no serious side effects questions have recently been raised as to whether the colorants are in fact inocuous. The purpose of the current study was to examine the effects which flourescein dyes have on neuronal physiology. The flourescein analogs were applied by bath perfusion to the isolated buccal ganglion of the marine mollusc, Navanax inermis, while the membrane potential and resistance of identified neurons were monitored with intracellular microelectrodes. Application of the dyes initiated a rapid increase in the membrane potential and conductance of the neurons. The changes in potential and conductance were a function of the particular dye and its concentration. The concentration required to produce half the maximum change in membrane potential, C_{50}, ranged from 10 micromolar for Phloxine B to greater than 100 millimolar for Flourescein. The C_{50} for Erythrosin B (F D & C Red No. 3), the only analogue approved for use in foods, was about 200 micromolar. Although the immediate effects of the dyes on membrane potential and conductance were reversible by washing with dye-free medium, several of the dyes also initiated a slow irreversible decline in the resting membrane potential. The rapid increase in membrane potential and conductance was apparently due to an increase in the potassium conductance of the membrane relative to other ions. The ability of the dyes to alter membrane potential was highly correlated with their octanol-water partition coefficient, increasing with hydrophobicity. These results suggest that these food and drug additives may have other biological activities, whose nature and intensity may be predictable on the basis of their partition coefficient. (Supported by NSF grant GB-43141)

590 INTRAMEMBRANE CHANGES IN PHOTORECEPTOR PEDICLES DURING RIBBON SYNAPSE FORMATION. Barbara J. McLaughlin and T. S. Reese. Dept. Anat., Univ. of Tenn. CHS, Memphis, TN 38163 and LNNS, NINCDS, NIH, Bethesda, MD 20014.

The outer plexiform layer of the developing chick retina from 11 embryonic days to 1-1/2 weeks post-hatching was studied with the freeze-fracture technique in order to characterize changes in the membrane structure of receptor terminals during synaptogenesis. A few particles were scattered on the cytoplasmic half of the undifferentiated pedicle membrane early in development. Later, as the pedicles extended filopodia, numerous small patches of large particles appeared between and on filopodial surfaces. Many patches were associated with craters, which were seen in cross-fractures through the underlying cytoplasm to correspond to plasmalemmal invaginations. Other particle aggregates, distinguished from the patches by their size and packing, resembled those at gap junctions; these were found throughout the rest of development. At later stages, when synaptic ribbons first appear, the patches were usually not associated with craters but were located close to neighboring patches and often aligned in long arrays on ridges in the pedicle membrane. Cross-fractures through these regions revealed synaptic ribbons adjacent to these arrays. Near maturity, when light responses appear, the number of patches decreased and the length and number of long arrays increased. Numerous small vesicle sites of the type typically associated with synaptic vesicle fusion appeared at the periphery of the long arrays. These observations suggest that ribbon synapse formation begins when vesicles deliver patches of particles to the pedicle membrane. These patches coalesce to become aligned in long arrays overlying the ribbon specializations. If our reconstruction is correct, intramembrane components of ribbon synapses are assembled at appropriate sites prior to final positioning of the synaptic ribbon.

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591 PHASE TRANSITIONS IN MYELIN MEMBRANES: Rodman G. Miller (SPON: Karen Arms). Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, N.Y.

Myelin (rat optic nerve) prepared for freeze fracture with glutaraldehyde fixation followed by glycerin impregnation demonstrates regions of the fracture plane which are free of particles surrounded by regions which are particle rich (see Pinto da Silva and Miller, PNAS, 72:4046, 1975). If the myelin is incubated (PBS with 20% glycerin and 1% glutaraldehyde) in the cold (6°C) for up a day, the particle free regions are flat and featureless. If incubation is carried out for longer than two days, crystalline patterns appear on the particle free regions. Often densely aggregated particulate regions (.1 - .5 μ dia.) are seen in these replicas. Formation of the cyrstalline patterns is insensitive to incubation with butylated hydroxytoluene or digitonin. The crystalline patterns are not seen when the incubation is carried out at 37°C or 21°C. Additional incubation for an hour at 37°C after incubation in the cold for 3 days destroys the crystalline patterns.

These results are consistant with a model of myelin membranes in which proteins bind specific lipids which coaggregate with the proteins during fixation and impregnation, creating cholesterol rich particle free regions (neurokeratin formation). Upon incubation at 6°C the protein specific lipids diffuse into the particle free regions and cholesterol and possibly other lipids diffuse into the particle rich regions. In the particle free regions, lipids which are below their phase transition temperature create the crystalline pattern seen.

592 INDUCTION OF ALL-OR-NONE ELECTROGENESIS IN CRAYFISH SLOW MUSCLE BY UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION. William Moody*(Spon. D. Kennedy) Dept. Biol. Sci., Stanford Univ., Stanford, CA. 94305

Electrical properties of tonic flexor muscle fibers of crayfish (P. clarkii) were studied after treatment with the uncouplers 2,4-dinotrophenol ($2X10^{-4}M$), dicoumarol ($10^{-5}M$) or carbonyl cyanide-m-chlorophenylhydrazone ($10^{-6}M$), or the inhibitor of oxidative phosphorylation oligomycin ($20\mu g/ml.$) Normal fibers respond to depolarization passively or with small graded responses. After treatment with the above drugs virtually all fibers generate overshooting, all-or-none action potentials. This increase in electrical excitability occurs gradually over the course of 1-5 hrs; the time course is dependent both on the compound used and temperature. Action potentials in treated fibers are unaffected by removal of external sodium or by tetrodotoxin ($4X10^{-6}g/ml.$), but are completely blocked by 2mM La^{+3}. Spiking fibers are capable of repeated contraction-relaxation when stimulated; the effect of the uncouplers is not accompanied by visible contracture of the fibers, or by any consistent change in input resistance or resting potential. Cyanide (2mM) markedly accelerates, and iodoacetate ($10^{-4}M$), an inhibitor of glycolysis, virtually blocks the action of dicoumarol; neither of these inhibitors alone mimic the effects of the uncouplers. Data demonstrating the iodoacetate block of the dicoumarol effect were collected only from fibers showing normal resting potentials and input resistances. These data indicate the possibility of a direct interaction between the rate of anaerobic glycolysis and the electrical excitability of the fiber membrane.

593 EFFECTS OF PREPULSES ON K^+ KINETICS IN MYXICOLA GIANT AXONS. T. L. Pencek* and C. L. Schauf. Departments of Neurological Sciences and Physiology, Rush Medical College, Chicago, Illinois, 60612.

In voltage-clamped Rana nodes Palti, Ganot, and Stämpfli (Biophys. J. 16: 261, 1976) found that potassium time constants (τ_n) during a fixed test pulse were consistently an order of magnitude smaller if the test pulse were preceeded by a more depolarized prepulse ($V_{pp} > V_t$) than if the prepulse were less depolarized ($V_{pp} < V_t$). This difference in τ_n was independent of the choice of x in the expression $G_k = \bar{G}_k n^x$, and was observed in normal and 80mM K^+ Ringer. Finally there seemed to be an effect of prepulse potential on the steady-state I_k during the test pulse.

None of this behavior seems to consistently exist in Myxicola axons in normal sea water or in high $[K^+]$. In the first series of experiments axons were held at potentials from -70mv to -60mv in ASW. Potassium currents at a variety of test pulses between -40mv and +60mv were then recorded following either no prepulse or 10-100msec prepulses from -160mv to +100mv. In the majority of cases (25 experiments) values for τ_n were independent of prepulse amplitude or duration. In four cases there was a significant decrease in τ_n for $V_{pp} > V_t$, but the magnitude was less than 40%. In one case there was an increase in τ_n for $V_{pp} > V_t$. We observed no detectible effect of $[K^+]$ on the magnitude of τ_n for either range of prepulse potentials used, and values for the steady-state K^+ current during V_t were consistently independent of prepulse potential and duration as well over the entire range of test potentials used. We conclude there is at present no compelling reason to modify existing descriptions of K^+ channel kinetics in Myxicola. (Supported by the Morris Multiple Sclerosis Research Fund and by NIH RCDA 1 K04 NS 00004-02 to C.L.S.)

594 QUININE AND Ca^{2+}-ACTIVATED K^+ CONDUCTANCE OF SPINAL MOTONEURONS. E. Puil and K. Krnjević, Centre de Recherches en Sciences Neurologiques, Université de Montréal and Department of Anaesthesia Research, McGill Univ., Montreal, Canada.

Armando-Hardy *et al.*, 1975 (*J. Physiol. 250*, 32P) have recently found that, of many agents tested, only quinine and quinidine selectively inhibited the K permeability of human red blood cells activated by internal Ca^{2+}. As these authors point out, it would be of interest to know whether comparable Ca^{2+}-activated K^+ channels in other types of cells are blocked by the same agents. In experiments on cats under Dial anaesthesia, we have examined the effects of extracellular iontophoretic administrations of quinine (released from 50 mM solutions of quinine SO_4 in HCl, pH 3.3) on lumbo-sacral motoneurons. In doses of 84-300 nA, quinine regularly caused a prolonged rise in input resistance and some depolarization, as well as a marked diminution or disappearance of the conductance increase associated with the post-spike hyperpolarization. These effects were only slowly and imperfectly reversible. They are fully consistent with a primary block of K^+ conductance, and confirm previous evidence that Ca^{2+}-activated channels contribute significantly to the resting K^+ conductance (Krnjević *et al.*, 1976, *Can. J. Physiol. Pharmacol. 54*, 73). An additional action of quinine, a temporary block of soma-dendritic action potentials, may be accounted for by some depression of depolarization-induced Na conductance. Quinidine, applied in the same way, produced comparable effects. These observations suggest that Ca-activated K^+ channels in widely different cells may be essentially similar. Supported by the Medical Research Council of Canada.

595 NERVOUS IMPULSES ARISING IN MID ROOT OF SPINAL ROOT AXONS OF DYSTROPHIC MICE. Michael Rasminsky* (SPON: P.E. Braun). Division of Neurology, Montreal General Hospital, Montreal, Canada.

In lumbosacral spinal roots of dystrophic mice, large diameter (up to 6μ) axons are bare at the mid root level but myelinated near the spinal cord and near their exit from the spinal canal. Contiguous portions of single ventral root (VR) fibers sustain both continuous and saltatory conduction, continuous conduction presumably occurring in the portion of the fiber lacking myelin (Rasminsky & Kearney, Neurology 26: 367, 1976).

Spontaneous activity on intact lumbosacral VRs was recorded biphasically (electrode separation < 1 mm); the initial deflection of each action potential indicated whether the impulse was travelling away from or towards the spinal cord (orthodromically or antidromically).

In normal VRs, spontaneous activity was orthodromic at all points on the root. In VRs of dystrophic mice (129B6F1/J dydy) spontaneous activity near the exit from the spinal canal was entirely orthodromic but was predominantly antidromic near the spinal cord. Following application of local anesthetic to the VR at its emergence from the spinal cord, spontaneous activity near the spinal cord became entirely antidromic but orthodromic activity was still observed more distally on the root. This indicated that impulses arose spontaneously in VR fibers in mid root and propagated in both directions. Simultaneous recording at two sites on the VR for cross correlation between antidromic activity at the proximal site and orthodromic activity at the more distal site indicated that the site of initiation of these impulses was usually several mm from the spinal cord. This is the region of the root where axons lose Schwann cell investment and become bare. The spontaneous activity observed in VR fibers of dystrophic mice must arise at or near this transition zone.

596 THE EFFECT OF ION PUMP POISONS ON THRESHOLD CURVES OF FROG SCIATIC NERVE AXONS. Stephen A. Raymond* Research Laboratory of Electronics, M.I.T., Cambridge, MA. 02139.

Threshold curves show the threshold for excitation for single fibers as a function of time. For frog sciatic axons the threshold curves following activity have a consistent form that can be divided into 5 sections: declining refractoriness, growing superexcitability, waning superexcitability, increasing depression, and declining depression. Using intermittent conditioning bursts one can establish and monitor an equilibrium depression level. Not only do ouabain and strophanthidin completely counter the buildup of depression with activity, they also eliminate equilibrium depression. The threshold drops below the control level for resting nerve, descending to the level reached during maximum superexcitability in unpoisoned axon. At intervals of 10-30 msec after a conditioning impulse, when the membrane is normally near peak superexcitability, the threshold of poisoned axons transiently shows a slight depression. The processes that make an unpoisoned axon superexcitable seem to make the poisoned one briefly depressed. With irreversible poisoning the threshold does not remain at the superexcitable level. After a period that is shorter with higher activity rates, the threshold climbs again to a point where the axon is unresponsive. Throughout the climb the superexcitable interval shows a threshold that is slightly reversed (less excitable) in comparison to that measured during intervals corresponding to depression. Anodal pulses successfully stimulate the axon even after cathodal pulses have begun to fail. This suggests the secondary increase in threshold stems from Na^+ depolarization of the axon. The results support the notions that an electrogenic Na^+ ion pump causes activity induced depression and that superexcitability is from an independent process.

597 STRUCTURE OF PLASMALEMMAL SURFACES DURING SYNAPTOGENESIS IN TISSUE CULTURE. Rosemary P. Rees. LNNS, NINCDS, NIH, Bethesda, Maryland 20014.

Isolated superior cervical ganglion neurons from 18 day fetal rats were established in culture and segments of 15.5 day fetal rat spinal cord were added 48 hours later. Tannic acid was applied after routine aldehyde and osmium fixation, according to Simionescu et al, J. Cell Biol., 1976, to stain membrane surface material during subsequent stages of synaptogenesis. Filamentous membrane material coated the outer surfaces of all neurons, fibroblasts, and satellite cells but its quantity and distribution varied. The filaments were 75-150Å long and spaced irregularly over the external surface. Somal and axonal plasma membranes of neurons had more filamentous coat than growth cones or mound areas and even more filamentous material marked regions where coated vesicles in neurons were open at the plasmalemma. At appositions between cells, actual contact between the adjacent outer dense laminae of the plasmalemmas never occurred. The filamentous coat was arranged along the apposed membranes in the same irregular pattern found elsewhere on the plasmalemma and frequently it contacted coat material on the adjacent plasmalemma. Where apposed membranes were slightly closer, separated by a gap of approximately 150Å, the filaments interdigitated across the intercellular gap and dense material was evident in the adjacent cytoplasm. A dense lamina bisected the intercellular gap but it was too close to both membranes to represent the apposed tips of filaments. Such regions of membrane specialization were 0.2 to 0.4 μm long and were rare between neurites and fibroblasts and between processes in the neuritic network. However, they were common in areas of contact between spinal cord neurites and sympathetic ganglion somata and processes. Whether these specializations, recognized with the tannic acid stain, represent an initial adhesion site required in the formation of synaptic junctions remains to be determined.

598 MECHANISM OF ACCUMULATION OF INTRAMEMBRANOUS PARTICLES AT NODES OF RANVIER Jack Rosenbluth. Depts. of Physiology and Rehab. Medicine, N.Y.U. School of Medicine, New York, N.Y. 10016

Freeze-fractured specimens of frog brain reveal large particles in the outer leaflet of the axolemma at nodes of Ranvier in a concentration of ~1-2000/μ^2. These may represent ionophores in this electrically excitable membrane. Such particles are sparse in the internodal axolemma but occur in the narrow, helical groove in the outer leaflet of the paranodal axolemma between axoglial junctional regions. Particle accumulations also occur in widened regions of this groove and sometimes in the internodal axolemma immediately adjacent to the paranodal region as well. This distribution suggests a mechanism by which intramembranous particles may become concentrated at intervals along myelinated axons. Particles may form in the cell body, where the protein synthetic apparatus is located, be inserted into the cell membrane there and then move distally within the axolemma. However, in the region of the paranodal axoglial junction the axolemma may be structured in such a way as to impede particle movement. The primary pathway for intramembranous particles through the paranodal region may therefore be along the helical groove which faces the cleft separating successive layers of the terminal edge of the myelin sheath. Particles reaching the nodal axolemma by this route would tend to accumulate at the node because of the restriction on their more distal movement imposed by the equally narrow exit pathway through the paranodal axolemma distal to the node. Such a trapping mechanism would also account for particle accumulations in the widened portions of the paranodal groove and in the internode just proximal to the paranodal region. This mechanism for particle accumulation requires no cytoskeletal component and would tend to result in the accumulation of particles which are large relative to the width of the paranodal groove. (Supported by grant NS 07495 from the NIH.)

599 SPECIFIC MEMBRANE CAPACITANCE OF CRUSTACEAN SKELETAL MUSCLE FIBERS. Kenneth L. Rossner* and R. G. Sherman. Dept. of Biology, Clark Univ., Worcester, Ma., 01610.

Numerous studies of the specific membrane capacitance (Cm) of crustacean skeletal muscle fibers involving the use of square wave pulse analysis have yielded values several times greater than those obtained for amphibian and mammalian fibers. These measurements were based on a model of the muscle fiber as a cylinder and did not take into account the contribution to capacitance made by the various invaginated membrane components. Consequently, the total amount of membrane contributing to capacitance was underestimated which led to an overestimation of Cm. We have undertaken a stereological analysis of long and short sarcomere-length fibers in crayfish walking leg extensor muscle to determine the amount of invaginated membrane present. This should permit us to determine an accurate estimate of true Cm. Preliminary results indicate that the extent of membrane contributing to capacitance is underestimated by a factor of 21.3 in the short sarcomere-length fibers (SSF) and 19.1 in the long sarcomere-length fibers (LSF) when only the surface membrane is considered. For example, our square wave pulse analysis of these fibers resulted in a Cm of 48 $\mu F/cm^2$ for the SSF and 32 $\mu F/cm^2$ for the LSF when only the surface membrane area was used in the calculations. Inclusion of our estimates of invaginated membrane in the calculations gave values of 2.3 $\mu F/cm^2$ for the SSF and 1.7 $\mu F/cm^2$ for the LSF. These values are comparable to those obtained for vertebrate fibers which are not extensively invaginated by membrane continuous with the sarcolemma. Therefore, crustacean muscle fibers do not appear to be appreciably different from vertebrate fibers with respect to their Cm.

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600 RADIOACTIVE BATRACHOTOXIN-DERIVATIVES AS PROBES FOR SODIUM CHANNEL COMPONENTS IN ELECTROGENIC MEMBRANES. H. Sundermeir*[1], J.O. Dolly*[1] E.X. Albuquerque[1], G. Brown*[2], W. Burgermeister*[2], J. Daly*[2] and B. Witkop*[2]. (SPON: W.D. Blake) Dept. Pharmacol. Exp. Ther.[1], Sch. Med., Univ of MD, Baltimore, MD. 21201 & Lab. Chemistry[2], NIH, Bethesda, MD. 20014.

Batrachotoxin (BTX), a steroidal alkaloid from the skin of the Colombian arrow-poison frog Phyllobates aurotaenia, has been shown to cause an irreversible depolarization of many electrogenic membranes by specifically increasing the resting sodium permeability at nanomolar concentrations. The BTX-treated membrane, when repolarized, is still capable of undergoing a normal transient sodium conductance associated with an action potential. These results inspired the preparation of tritiated-BTX as a probe for quantitation and characterization of the macromolecular component(s) of the sodium channel. BTX 20α-[^{3}H]-benzoate (18 Ci/mmole) was prepared from tritiated benzoic acid and batrachotoxinin A via anhydride coupling. BTX-benzoate (80 nM at 37°C) displayed the same ability as native BTX to depolarize rat phrenic nerve-diaphragm preparations and the LD50 values of BTX and BTX-benzoate SC in mice were similar. Preliminary binding studies, using both centrifugation and filter assays, showed that the radioactive toxins bind to garfish olfactory nerve (whole nerve, nerve-homogenate and axonal membrane fraction). The density of specific sites is less than that obtained with tetrodotoxin. (Supported in part by USPHS grant NS-12063.)

601 BIOSYNTHESIS OF POLYPRENOL-LINKED SUGARS BY MYELIN- AND AXOLEMMA-ENRICHED MEMBRANE FRACTIONS. C.J. Waechter*, J.B. Harford* and G.H. DeVries. Dept. Biochem. Univ. Md. Sch. Med., Baltimore, Md. 21201, and Dept. Biochem. Med. Coll. Va., Richmond, Va. 23298.

Previous studies have revealed that mannosylphosphoryldolichol (MPD) participates in the assembly of some membrane glycoproteins in calf white matter [Waechter et al., Arch. Biochem. Biophys., 1976, in press]. We have speculated that if glycosyltransferases were to play a role in the myelination of axons, perhaps dolichol-linked sugars provided the activated glycosyl groups at the cell surface. In this study we have found that bovine myelin- and axolemma-enriched (AL) fractions obtained from purified myelinated axons [DeVries et al., Exp. Brain Res. 23, 236, 1975] catalyzed the transfer of [^{14}C]mannose from GDP-[^{14}C]mannose into mannolipid. The mannolipid formed in the presence of exogenous dolichol monophosphate (DOLp) is chemically and chromatographically identical to MPD. In the absence of exogenous DOLp the amount of mannolipid formed by the myelin-enriched fraction is slightly higher than the AL fraction. However, in the presence of exogenous DOLp, MPD formation by the AL fraction was stimulated seventy-fold while mannolipid formation by the myelin-enriched fraction was increased only seven-fold. These results are consistent with the conclusion that the AL fraction contains higher levels of MPD synthase, but have a lower content of endogenous DOLp. The AL fraction also catalyzed the transfer of N-acetyl [^{14}C]glucosamine from UDP-N-acetyl [^{14}C] glucosamine into lipid. The formation of N-acetylglucosaminyl lipid was stimulated by DOLp.

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602 DIFFERENCES BETWEEN CURARE BINDING ASSAYS IN LIVING AND DEAD ANIMALS. David C. Wood and Charles See*. Psychobiology Prgm., U. of Pittsburgh, Pittsburgh, Pa. 15260.

d-Tubocurarine and other nicotinic blocking agents bind to the membrane of the protozoan, Stentor coeruleus, and specifically desensitize the animals to mechanical stimuli by reducing the transduction of mechanical stimuli into receptor potentials. Two assays for the binding sites of radioactively labelled d-tubocurarine were developed: one involving the incubation of living animals while in the second animals were killed by fixing them to a glass fiber filter disc prior to incubation. These procedures produce saturation curves with different K_d's: 2.9×10^{-4}M for the live animal assay and 9.6×10^{-6}M for the dead animal assay. Data derived from the 2 assays was compared to dose-response data for the sensitivity of stentor to mechanical stimuli. The correlation between the live animal saturation curve and the behavior data was 0.99 while for the dead animal saturation curve it was 0.966. Correlations were also obtained between behavioral and assay data while incubation time and the presence of competing ligands was varied. In all cases the correlation between the behavioral data and the live animal assay data was higher. From these results it is concluded that ligand binding studies employing homeogenized or otherwise disrupted tissues may be studying binding to sites whose properties have been altered by the preparative procedure so that they no longer reflect the properties of the binding site in its physiologically active form.

603 FINE STRUCTURAL LOCALIZATION OF THE NA+, K+ ACTIVATED ATPase IN KNIFEFISH BRAIN. John G. Wood, D. H. Jean, J. N. Whitaker, Barbara J. McLaughlin and R. Wayne Albers. Dept. of Anat. and Memphis V. A. Hospital, Univ. Tenn. Center for Health Sciences and Laboratory of Neurochem. NINCDS. Bethesda.

Rabbit antisera to the Na+, K+-activated ATPase purified from electric eel(Electrophorus) have been used in immunocytochemical experiments to localize the whole enzyme in the brain of the South American knifefish (Sternarchus albifrons). Knifefish are perfused through the heart with a fixative containing 0.1% glutaraldehyde and 4.0% paraformaldehyde. The brain is removed and stored overnight in 4.0% paraformaldehyde and 50-75 μ slices of optic tectum and cerebellum are obtained for immunocytochemistry. The slices are treated with rabbit antisera to the ATPase followed by peroxidase conjugated fractions of goat anti-rabbit serum. At the electron microscopic level the reaction product for peroxidase reveals that the enzyme is located on the plasma membranes of the somata and dendrites of neurons and on the somata and cellular processes of glia. Staining of the oligodendroglial plasma membrane extends to myelinated axons where the staining pattern exactly follows the outermost edge of the compacted myelin lamellae. At the node of Ranvier the enzyme is localized along those portions of the axolemma not covered by the myelin sheath. The region of the axolemma covered by the myelin is never observed to be labeled even in severely disrupted tissue in which the reagents should have free access to membrane. The results suggest the sequestering of a protein critical to nerve function only along regions of the axonal membrane of myelinated axons where the enzyme activity of the protein is needed. The capacity of membranes to restrict mobility of proteins should be considered in models of membrane structure in which lateral mobility of membrane components is considered a major characteristic. USPHS 5T01-GM-00202, NS-12590; VA; Sloan Foundation.

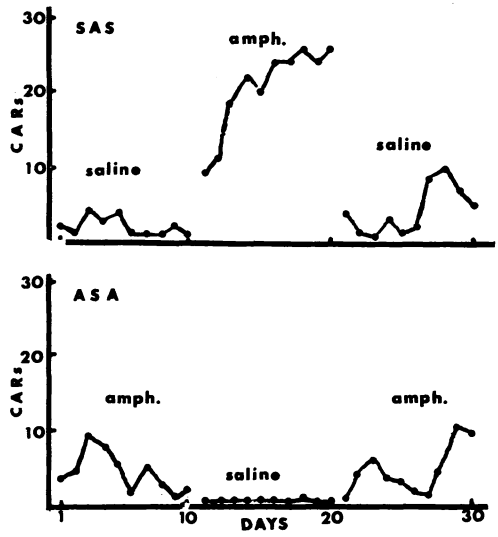
Memory and Learning

604 D-AMPHETAMINE FACILITATION OF CONDITIONED AVOIDANCE RESPONSE PERFORMANCE IN RATS WITH ISOCORTICAL ABLATIONS. M. S. Beattie, T. S. Gray*, J. R. Rosenfield*, P. M. Meyer and D. R. Meyer. Lab. Comp. and Physiol. Psych., Ohio State Univ., Columbus, OH. 43212.

Near total removal of the neocortex in rats severely disrupts the acquisition of a two-way conditioned avoidance response (CAR) (Meyer, Johnson and Vaughn, Brain Res., 22: 113-120, 1970). In the present study, daily pre-training injections of d-amphetamine sulfate (1 mg/kg) were found to be effective in reducing the CAR deficit in such preparations.

Two groups of decorticated rats were trained for 30 days (30 trials/day) in a shuttle box, with a buzzer serving as the conditioned stimulus (CS). Group SAS (n=11) received saline injections 30 min prior to each of the first 10 training sessions, amphetamine prior to training for the next 10 days, and saline before each of the last 10 daily sessions. Group ASA (n=12) received amphetamine for the first 10 days, saline for the next 10 days, and was returned to amphetamine for the last 10 training sessions.

Figure 1 shows the median number of CARS for SAS and ASA rats over the course of training. CAR performance for both groups was significantly better under amphetamine conditions than under saline conditions. However, this facilitation was most dramatic for Group SAS, which had received 10 days of training with saline before being trained with amphetamine. ASA rats failed to exhibit such high levels of performance during either initial amphetamine training or during amphetamine training following interposed saline training. Thus, the degree of CAR facilitation induced by amphetamine seems to depend upon whether initial training on the habit is conducted without the drug. For both groups, amphetamine facilitation was highly state dependent; i.e., a return to saline after drug treatment resulted in a significant decline in performance.



Not only did amphetamine increase the number of avoidance responses, but, on those trials on which the animals failed to avoid, it also increased locomotor activity in response to both the CS and to footshock. Since locomotor activity is an essential component of the active avoidance situation, an increase in locomotor tendencies could be another important variable in the ameliorative effects of amphetamine on CAR performance in rats with extensive neocortical ablations.

The results of the present study suggest that the CAR impairments seen in decorticated rats under saline training conditions reflect an inability to respond appropriately to the fear-eliciting CS rather than a deficit in associative mechanisms per se, and provide additional evidence that subcortical structures are capable of sustaining relatively complex learned behaviors.

605 RETROGRADE AMNESIA INDUCED IN CHICKS BY L-PROLINE IS STEREOSPECIFIC.
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L-Proline (L-PRO) impairs memory retention when injected shortly after one-trial avoidance training of neonatal chicks, as compared with uninjected controls or controls injected with L-isoleucine (L-ILE) [Van Harreveld and Fifkova, Brain Res. 81, 455, 1974; Cherkin, Eckardt and Gerbrandt, Science, in press, 1976]. Among the known chemical amnesic agents, L-PRO is unique in its freedom from confounding obvious side effects, including gross EEG changes known to be associated with amnesia, motor convulsions, unconciousness, illness, and appreciable mortality rates. This complexity reduction favors identification of the specific memory-processing mechanism(s) presumably impaired by retrograde amnesic treatments.

To test the stereospecificity of the L-PRO amnesic effect, we made direct comparisons of L-PRO with D-PRO. In Exp. I, we injected 44-hr old chicks intracerebrally with 10 µl per hemisphere of isotonic L- or D-PRO (300 mM, pH 7.2±0.2), 1 min after a 10-sec one-trial training of the chicks to suppress their "innate" peck response to a small shiny bead. Peck suppression, with avoidance of the normally attractive bead, was conditioned by coating the bead with an aversive liquid (methyl anthranilate) immediately prior to the training trial. Retention of the avoidance response was tested 24 hr later using the dry uncoated bead; reduced avoidance scores (increased pecking) indicate impaired memory retention. In Exp. II, we increased the concentration of L- and D-PRO to 600 mM and we also evaluated the retrograde amnesic effect by adding groups injected 255 min after training (Exp. II-2).

The results (see Table) confirm that L-PRO is an effective amnesic agent and that D-PRO has no more effect than had L-ILE, previously shown to be ineffective (Exp. III). When the injections were delayed for 255 min after training, the two stereoisomers gave nearly identical scores, in contrast to the significant differences observed when the injections were made 1 min after training. This confirms the retrograde nature of the L-PRO effect. Furthermore, the 255-min score for the L-PRO group did not differ significantly from control levels, thus confirming the absence of deficits in peck performance or in retrieval in L-PRO-injected chicks.

Exp.	Injection	Conc (mM)	TII[a] (min)	N	Avoidance Score (%)[b]	χ^2	p
I	L-PRO	300	1	39	30.8	4.73	<0.04
	D-PRO	300	1	40	55.0		
II-1	L-PRO	600	1	40	40.0	5.20	<0.03
	D-PRO	600	1	38	65.8		
II-2	L-PRO	600	255	40	52.5	0.01	>0.90
	D-PRO	600	255	39	53.8		
III[c]	L-PRO	300	1	103	36.9	11.62	<0.001
	L-ILE	300	1	104	60.6		

[a]TII = interval between training and injection.
[b]Percentage of chicks which avoided pecking the bead for 10 sec.
[c]Previous experiment, under identical conditions [Cherkin et al., Science, in press, 1976].

Comparisons by two other measures (latency of peck response; peck rate) led to the same conclusions. The natural L-configuration appears to be required for the observed amnesic effect of PRO.

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606 IN VITRO INCORPORATION OF URIDINE TRIPHOSPHATE INTO NUCLEI OF TRAINED AND CONTROL MICE. Patric A. Clapshaw*, and Kurt Schlesinger* (SPON: M. E. Dubin). Dept. Psych., Univ. of Colorado, Boulder, Co. 80309.

The incorporation of radioactive uridine triphosphate (UTP) into precipitable counts was measured in several experiments in which animals were trained to avoid an electric shock. In the first experiment, animals were trained to jump onto an escape shelf during the presentation of the conditioned stimulus, a light and buzzer. Yoked control and quiet control mice were used. The trained animals reached criterion, 7 out of 10 consecutive correct responses, in an average of 20.5 trials. Yoked control animals, trained to the same criterion on the second day of the experiment, reached the criterion in 12.9 trials; the conditioned animals, when retrained on the second day, reached the criterion in 12.5 trials. This suggests that yoked control animals in this paradigm are learning something, probably the contingency between the conditioned stimulus and the shock. For this reason, another learning task was developed; here, animals turned a wheel during the presentation of a conditioned stimulus, a tone, to avoid an electric shock. Yoked control animals were given the tone randomly, but were yoked to the experimental animal with respect to the electric shock. Trained animals took an average of 20.5 trials to reach criterion of 7 out of 10 consecutive correct responses; when retested on the second day of the experiment these animals reached the criterion in 12.6 trials. The yoked control animals, when trained on the second day, required 20.8 trials to reach criterion. Therefore, the yoked control animals in this situation, showed no evidence of learning.

Trained and yoked control animals, from this second experiment, were used in an in vitro transcription assay performed on isolated whole brain nuclei. These nuclei were incubated with specific concentrations of radioactive UTP, and precipitable counts were collected at 10 and 20 minutes. No significant differences in precipitable counts were observed between nuclei obtained from trained and yoked control mice. When these groups were compared with nuclei obtained from quiet control animals, more counts were observed in the nuclei of the quiet mice, although this difference was not statistically significant. When the number of wheel turns was used as a measure of motor activity, a correlation of $-.52$ was obtained between this measure and counts precipitated from the nuclei of trained and yoked animals.

In experiments currently in progress we are measuring the availability of UTP to these nuclei during the incubation. We will use these data as a correction factor to estimate the relative rate of incorporation of UTP into precipitable counts.

Motor activity and precursor pools have long been problems in research on RNA and learning or memory. Further experiments are in progress to assess whether residual increases in RNA synthesis occur as a function of training, after the effects of motor activity and precursor pool fluctuations are taken into account.

607 NICTITATING MEMBRANE CONDITIONING TO TONE IN IMMOBILIZED ALBINO RABBITS. John F. Disterhoft and Helen H. Kwan*. Dept. Anatomy, Northwestern Univ. Med. Sch., Chicago, IL. 60611.

The acquisition of nictitating membrane conditioned responses (CRs) in young adult, male albino rabbits to a tone conditioned stimulus (CS) paired with corneal air puff (US) was examined. The animals' heads were immobilized by 4 restraining bolts in a modified stereotaxic; their bodies were placed in a canvas sack to minimize movement. In conditioning experiments, a 425 msec, 85 db (re 0.0002 dyne/cm^2), 1000 Hz tone (CS) was paired with a 5 psi, 200 msec puff of compressed nitrogen (US) presented to the rear of the left cornea. A 75 db background white noise was present. The intertrial interval averaged 70 sec. In pseudoconditioning experiments, the tone and puff were independently presented in pseudorandom order at a 35 sec average intertrial interval. Nictitating membrane closure was measured with an integrated circuit light reflection transducer. Eighty trial sessions were given on 3 successive days. In 14 conditioned and 5 pseudoconditioned rabbits, nictitating membrane responses were recorded from only the puff (left) eye. Recordings were made from both eyes in an additional 8 conditioned and 4 pseudoconditioned rabbits.

All 22 conditioned rabbits showed CR acquisition in the puff eye during Training Session I. The trial of the first CR varied from trial 4 to trial 49. The group learning curve showed a monotonic increase from trial 11 to 40 and asymptoted near 75% in the last 40 trials. During Sessions II and III the curve asymptoted above 90% and showed 100% responses on 36 of 80 trials in Session III. The average response latency decreased significantly from 177 msec in Session I to 135 msec in Session II. CRs were larger in Sessions II and III. The acquisition function was also studied with the trial of the 10th CR as the anchor point. An abrupt shift was visible between trials -12 and -11. The mean probability of response increased 48% on trials -11 to -7 compared to trials -16 to -12. Pseudoconditioned rabbits showed almost no CRs in any session.

No CRs were detected in the non-puff eye in Session I. Only two of the 8 rabbits showed more than 9 non-puff eye CR's in Sessions II or III. In one of these two, they disappeared in Session III. Non-puff CR's, when present, were smaller and of longer latency than puff CR's. Commonly, non-puff eye UCRs were present at the latency of the puff eye UCRs.

These data indicate that nictitating membrane conditioning to tone in albino rabbits is a useful preparation in which to examine the neurophysiological substrates of conditioning. Acquisition occurs in immobilized rabbits within 1½ hours. The CRs are well defined behaviorally. The acquisition function for individual animals, and for the group, has a sharp increase between 12 and 11 trials before the trial of the 10th CR. Averaging in relation to the 10th CR should facilitate detection of neural events preceding behavioral acquisition. The fact that the behavioral response is primarily unilateral, although the tone CS is bilateral, offers a within-subject control for studies of neurophysiological or neuroanatomical correlates of conditioning at the efferent neurons. (Supported by NIH Grant 1 R01 NS12317.)

608 PREFRONTAL UNIT ACTIVITY DURING RETENTION OF SPATIAL AND NONSPATIAL INFORMATION. Joaquin M. Fuster and Richard H. Bauer. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024.

Cooling the cortex in the area of the sulcus principalis produces a reversible deficit in the monkey's performance of tasks requiring short-term retention of sensory information, whether this information is defined by spatial relationships or not (Bauer and Fuster, 1976). The deficit is critically dependent on retention time, namely, the length of delay interposed between a stimulus and a behavioral choice contingent upon it. This temporal dependency of the deficit supports the mnemonic hypothesis of prefrontal function. The present study focuses primarily on the discharge of prefrontal cortex cells during retention of both spatial and nonspatial memoranda.

Two monkeys were trained to perform a delayed matching-to-sample (DMS) task and a spatial delayed response (DR) task on a panel with three stimulus-response keys forming an isosceles triangle. In DMS, the stimulus or sample was a color, red or green, displayed on the top key and terminated by the animal's pressing of the key. After a delay, both colors appeared on the lower keys and the animal was then rewarded for choosing the sample color. In DR, the stimulus was a positional cue: white light in one of the two lower keys, right or left. After a delay, both keys were lit. Reward resulted from choice of the previously lit key. The color on each key (in DMS) and the position of the cue (in DR) were changed randomly between trials. In a testing session the animal was given several alternating blocks of trials of both tasks. Delays between 0 and 32 sec. were used.

Spike discharge was extracellularly recorded by roving microelectrode. This report is based on study of 157 units in the cortex of the depth and banks of the sulcus principalis; 57 were tested on DMS only, 13 on DR only, and 87 on both tasks. During presentation of the sample or cue, many units showed phasic firing-changes, mostly excitatory. During the delay--trials with 16 sec. delay were used while recording--, discharge frequency differed from inter-trial baseline in about one-half of all units; discharge was color-dependent in 15% of DMS-tested units and position-dependent in 21% of DR-tested units. Differences as a function of task were observed in 46 of the 87 units that were tested on both tasks. Fourteen units were predominantly or exclusively activated during DMS delays and 18 others during DR delays. Histological reconstruction revealed some clustering of units of similar type with respect to task or stimulus, but failed to show systematic differences of unit-type distribution between hemispheres or between parts of the cortical region explored.

The results suggest that certain neurons in the area of the sulcus principalis participate in the neural processes that allow temporary retention of sensory information. According to unit recordings, there seems to be considerable topographic overlap of cellular populations related to spatial and nonspatial task-performance. This study provides further support to a role of prefrontal cortex in dynamic memory.

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609 MEMORY PROCESSES DIRECTLY MODIFIABLE BY REINFORCEMENT.
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Since considerable evidence suggests that electrical brain activity is directly modifiable by operant conditioning, and since short-term memory processes are widely held to be coded in form of dynamic electrophysiological events, it follows that short-term memory processes, per se, can be conceptualized as operants, and thus be amenable to operant conditioning. Evidence for a direct influence of reinforcement on memory processes was obtained in the following experiments: One min. access to food given 50-60 sec after the footshock in a one-trial passive avoidance (step-down) situation facilitated learning of this task in mice ($p < 0.025$, t-test). Food reinforcement presented before or after this interval did not have this effect. To test the generality of this phenomenon rats were used in a second study. The reinforcer consisted of 30 sec of reinforcing stimulation of the lateral hypothalamus presented 30 sec after the footshock in a step-through avoidance reversal task. The stimulated rats learned better than the nonstimulated controls ($p < 0.05$, U-test). These results suggest that short-term memory processes are directly modifiable by reinforcement.

To test the possibility of a direct influence of punishment on memory processes a one-trial step-down avoidance task was used. Mice were given punishment (10 sec in ice water) either immediately, 30, 60 or 90 sec after the footshock. The groups given immediate or 60 sec delayed post-trial punishment showed a decrement in learning 24 hs later ($p < 0.05$), whereas the performance of the 30 and 90 sec delay groups did not differ from that of the controls.

Since electroconvulsive shock (ECS) has amnestic, but also punishing properties, we repeated the latter experiment but administered post-trial ECS instead of ice-water punishment. The results were virtually the same: ECS presented either immediately or 60 sec after the footshock had the most pronounced amnestic effects. ECS presented after 30 sec resulted in a weak, but nonsignificant decrement in learning, and the 90 sec delay group was not significantly different from the controls (pseudo-ECS).

610 SCOTOPHOBIN: STIMULUS SPECIFIC ENHANCEMENT OF ONE-TRIAL PASSIVE AVOIDANCE LEARNING. David H. Malin and Dinah Babcock*. Univ. of Houston at Clear Lake City, Houston, Tx. 77058.

Scotophobin is a peptide found in the brains of rats taught to fear a dark compartment. Injection of synthetic scotophobin into naive mice and fish has been reported to induce increased avoidance of dark compartments. However, injection into rats has increased avoidance only in rats that had received one mild, brief shock in the dark compartment. This suggested the possibility that scotophobin may non-specifically amplify or augment the fear produced by the shock rather than induce fear of a particular type of compartment. These alternatives were tested by observing the effects of synthetic scotophobin on one trial passive avoidance to white as well as black compartments.

Sixteen rats were given a single 0.25 ma, 1 second scrambled shock upon stepping through from an entrance platform into a white shock box, while another sixteen rats received the same treatment upon stepping into a black shock box. Half of each group was then injected with 38.5 ug/kg scotophobin (Synthesized by D. Desiderio) in distilled water while the other half received distilled water only. Beginning one day later, each rat was tested daily for four days for its approach latency to the same compartment in which it was shocked. The results, averaged over the four test days are summarized below.

Average Approach Latency in Seconds

	To White Box	To Black Box
Placebo	4.6	3.8
Scotophobin 38.5 ug/kg	6.1	15.8

Three way analysis of variance, repeated measures, (drug x test environment x days) revealed a significant ($p < .02$) interaction effect between drug and test environment - the effect of scotophobin on approach latencies depended significantly on which box was to be approached. According to Duncan's Range test, scotophobin significantly increased latencies in the black box condition ($p < .01$), but not in those animals tested with the white box. These results, together with previously published experiments, are consistant with Ungar's view that the effects of scotophobin resemble not generalized fear but a mild conditioned aversion to a dark compartment. Thus, the appearance of scotophobin in the brains of animals trained to such an aversion may have some connection with the process of memory formation for this learned response.

611 ORGANIZATION OF SHORT-TERM VERBAL MEMORY IN LANGUAGE AREAS OF HUMAN CORTEX: EVIDENCE FROM ELECTRICAL STIMULATION. George A. Ojemann. Dept. of Neurological Surgery, Univ. of Wa., Seattle, Wa. 98195.

There are a number of standard neurosurgical procedures that require electrical stimulation of the brain under local anesthesia, if operative risk is to be minimized. These occasions have been used to study the anatomic substrate for human short-term verbal memory (STVM), by observing the effects of the electrical stimulation of the therapeutically determined brain site on a standard STVM test, patterned after the single item paradigm of Peterson and Peterson (J. Exp. Psychol. 58: 193, 1959) modified by the use of consecutive trials, with naming of object pictures as input, a 6 sec. distraction (counting backward by 3's) and retrieval by cued recall (Ojemann et al., Brain 94: 225, 1971). Electrical stimulation (60Hz, 2.5 msec biphasic square waves, 4 sec trains) is applied during the input, or retrieval, or input and retrieval, or only the storage (distraction) phases of this test on randomly selected trials. Interspersed trials with no stimulation are used as controls.

Electrical stimulation of the lateral thalamus is required in stereotaxic thalamotomies for dyskinesias. The effects of this on the STVM test have been reported previously (At this society in 1971 and 1974, and Ojemann et al., Brain 94: 225, 1971; Ojemann, Brain and Language 2: 202, 1975). Stimulation of cortex, to identify language and motor areas, is also necessary in craniotomies for resection of epileptic foci in the temporal or frontal lobes of the language dominant (here left) hemisphere. The effects of this cortical stimulation on the STVM test were determined at 25 sites on lateral cortex of left posterior frontal, parietal and temporal lobes in 5 patients, using 4-10 mA currents. Each patient contributed 4-6 sites. The STVM test was very easy for these patients. Only one STVM error was made by any patient on non-stimulation control trials. Two findings emerged:

1. The cortical areas concerned with language are generally not involved in STVM. Language involvement of a cortical area was determined by evoked changes in object naming. From 9 sites no changes in either language or STVM were evoked. In 8 sites only changes in STVM were evoked, in 5 only language changes, and from only 3 sites were both language and STVM changes evoked. "Anomic" disturbances of object naming were evoked from 5 locations in the parietal-temporal lobe of 4 patients. The same current levels, in the same locations that evoked "anomia" on no occasion disturbed cued recall from STVM.

2. Different areas of the left frontal, parietal and lateral temporal cortex of man are related to different STVM processes. Stimulation during input or storage (distraction) phases of the STVM test disturbed STVM when the current was applied to parietal or temporal operculum, immediately anterior to the posterior language area. This effect was noted in all 3 patients so stimulated in this area. No change in the distracting task occurred during these stimulations. This type of response to stimulation suggests that this area may be the site of the active storage processes in STVM. Stimulation during retrieval disturbed STVM only from sites above the Sylvian fissure, predominately in posterior frontal lobe. These patterns of STVM changes evoked with cortical stimulation are not the same as those seen with left lateral thalamic stimulation.

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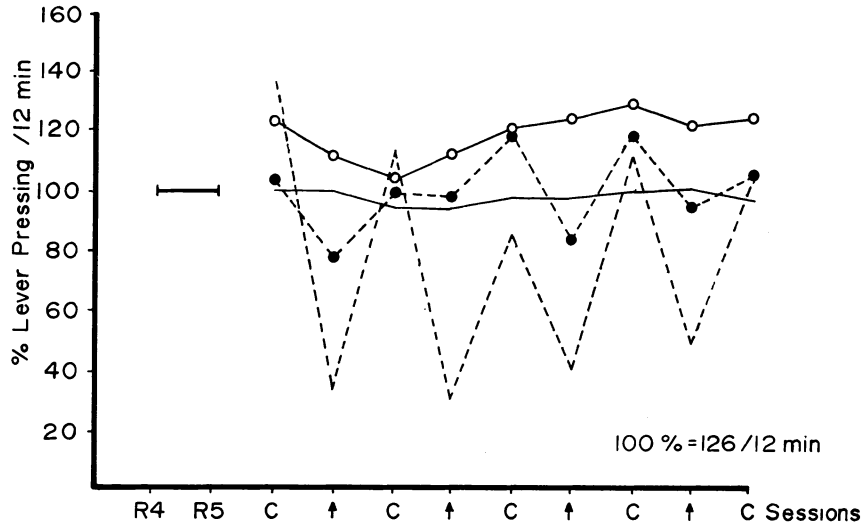
612 CHOLINERGIC BLOCKADE OF THE CAUDATE NUCLEUS: EFFECTS ON HIGH AND LOW DEGREES OF TRAINING. Roberto A. Prado-Alcalá and Guillermo G. Cobos-Zapiaín*. Physiol Dept., Med. Sch., Natnl. Univ. of Mexico, Ap. Postal 70250, Mexico 20, D. F., Mexico.

It has been shown that the functional integrity of the caudate nucleus (CN) is essential for the performance of instrumental conditioned responses, and that such behaviors are mediated by a cholinergic mechanism within this structure. In this paper we show that such cholinergic modulation is mainly involved when recently acquired behaviors are performed, since overtrained responses remain unaffected by a cholinergic blockade of the CN.

Cats were trained to lever press following a continuous reinforcement schedule, and were assigned to one of the following groups: I, trained for 15 sessions: a) unimplanted controls, b) implanted-NaCl, c) implanted-atropine; or II, trained for 30 sessions: d) implanted controls, e) implanted-NaCl, f) implanted-atropine. Except for the animals of group a, all were chronically implanted on day 16 (group I) or 30 (group II) with double-walled cannulae into the dorsal aspect of the CN. All animals were retrained for 6 days; 10 min before the 7th retraining session and on successive odd sessions bilateral microinjections (5 µl) of saline solution (groups b and e) or 80 µg of atropine sulphate (groups c and f) were delivered until 4 microinjections of each substance were completed, while groups a and d were trained as usual. On even sessions (control sessions) no microinjections were made.

No differences in performance were found among the groups during retraining and control sessions. On microinjection days, however, those animals that had been trained for 15 days and were injected with atropine showed a highly significant impairment in lever pressing. In contrast, no differences in instrumental behavior were found in any of the rest of the groups. (See figure).

We conclude that as training progresses either the functional role of cholinergic mechanisms within the CN becomes less involved in mediating instrumental conditioned responses, or that the caudate nucleus, as a whole, plays a less significant role in such processes.



Each point represents % lever pressing of each group. R4 and R5: 4th and 5th retraining sessions for all groups. C: control sessions; arrows: microinjections sessions. Fifteen-sessions groups:--- Atropine, —— NaCl. Thirty-sessions groups: •----• Atropine, o——o NaCl.

613 INTRACRANIAL L-PROLINE INDUCES RETROGRADE AMNESIA IN GOLDFISH. Walter H. Riege and Arthur Cherkin. Psychobiology Res. Lab., VA Hospital Sepulveda, CA 91343 and Dept. Psychiatry, Sch. Med., UCLA, Los Angeles, CA 90024.

Few treatments shown to interfere with formation of long-term memory are without obvious toxic side effects. L-Proline (L-PRO) at a nontoxic dose induces retrograde amnesia in chicks [Van Harreveld and Fifkova, Brain Res. 81, 455, 1974; Cherkin, Eckardt, and Gerbrandt, Science, in press]. In goldfish, we found the action of L-PRO to be temperature-dependent as well as time-dependent. Goldfish (N=982) were trained in one trial with brief electric shock (2.0 V/cm) to suppress their spontaneous upstream swimming into a quiet well. Memory retention one day later was not markedly influenced by the temperature (15°C, 22°C, or 29°C) at which they had been acclimated, trained (TR) and tested. Intracranial injection of L-PRO (1 µl, 300mM) before or after training (-16, +4, +16, or +64 min) led to amnesia in fish injected 4 or 16 min after training (Fig. 1). Low temperature lengthened and high temperature shortened this post-training interval for effective amnesic action of L-PRO. Control fish given needle puncture only or an injection of L-Isoleucine (1 µl, 300 mM), showed no impairment of one-day memory retention. Neither swimming nor feeding behavior of fish were altered by the injection of L-Proline.

(Supported by the Veterans Administration under Projects 1387-01 and 7452-01).

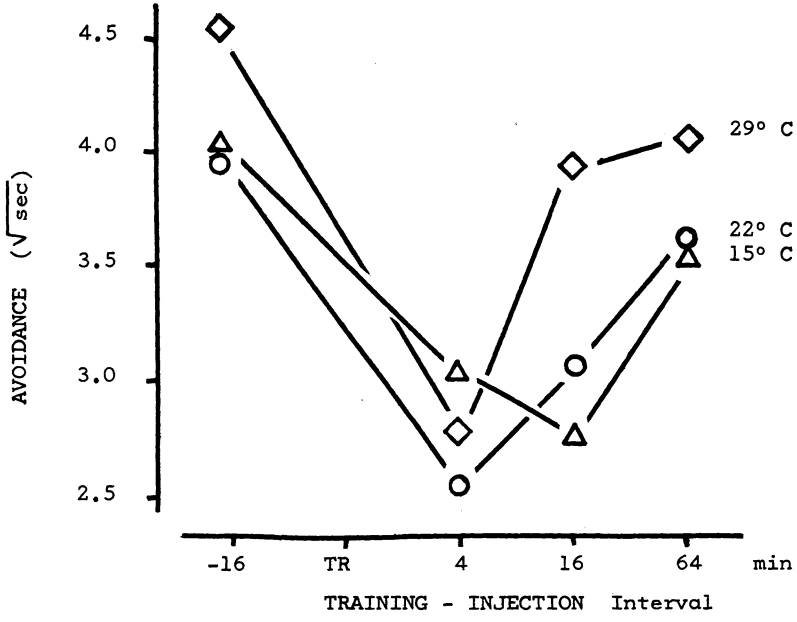


Fig. 1 One-day memory retention of goldfish (36/group) after one-trial avoidance training and injection of L-Proline.

614 DIFFERENTIAL PLASTICITY OF MORPHOLOGICALLY DISTINCT NEURON POPULATIONS IN THE MEDIAL GENICULATE BODY OF THE CAT DURING CLASSICAL CONDITIONING.
D. K. Ryugo* and N. M. Weinberger. Dept. Psychobiol., UCI, Irvine, CA. 92717.

In the continuation of our investigation of a possible role of the auditory system in the establishment of conditioned pupillary responses (Oleson et al., 1975), we were prompted to examine the relationship between morphology and neural plasticity in the medial geniculate body (MGB). The MGB of the cat has routinely been subdivided into dorsal (MGd), medial (MGm), and ventral (MGv) divisions (Morest, 1964; Ramon y Cajal, 1955). This architectonic parcellation has been confirmed by the differential spatial distribution of collicular afferents (Morest, 1965; Ryugo and Killackey, 1975) as well as the distinct type of thalamocortical projections emitted by each MGB component (Raczkowski et al., 1976). Given this contrast among MGB subdivisions, we believed that such morphological differences might underlie different functional roles during the acquisition of a behavioral conditioned response to an auditory conditioned stimulus (CS). Thus, we examined multiple-unit activity simultaneously in each of the MGB components of paralyzed cats during sensitization, conditioning, and discrimination training of the pupillary dilation response. White noise served as the CS+ (reinforced), tone as the CS- (non-reinforced) and forelimb shock as the US. The CS-US interval was one sec.

During sensitization (CS+, CS-, US presented randomly) neurons in all three regions responded to all stimuli, but only MGm neurons showed direct driving by the US. During conditioning only MGm neurons developed a systematic response enhancement to the CS+, relative to the responses during sensitization. The initial, short-latency onset excitation remained constant over the course of training; the enhancement was generated by the development of the later, sustained response. There was no change in MGv or MGd responsiveness during training to either the reinforced CS+ or the non-reinforced CS-. Discrimination training demonstrated that the behavioral and MGm neural response acquisition was specific to the CS+. However, analysis of the acquisition curves revealed that the conditioned behavior was attained sooner than the increases in MGm activity. Although the conditioned brain changes did not precede the acquisition of the pupillary response, we have morphologically and physiologically identified a site within the auditory thalamus that demonstrates the capacity to acquire a specific enhancement in responsiveness in conjunction with the development of conditioned pupillary behavior. Although the necessary and sufficient conditions for the development of neuronal conditioned responses cannot yet be completely specified, the present findings suggest a critical role for the nature of the initial neuronal response to the unconditioned stimulus.

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615 SYMBOL-DIGIT SUBSTITUTION PROCESSES IN CHRONIC APHASIA. Aaron Smith. Neuropsychological Laboratory, Dept. of Phys. Med. & Rehab., Univ. of Mich. Med. Sch., Ann Arbor, Michigan 48109.

Rejecting differentiations of Broca's motor, Wernicke's sensory, conduction and other presumably discrete aphasic syndromes hypothesized on anatomical bases, Hughlings Jackson and subsequent investigators called attention to the symbolic processes underlying all language functions. They suggested that despite various manifestations, aphasic disorders reflected a fundamental disturbance in the processing of verbal symbols that underlies expressive and receptive language functions. The development of the Symbol Digit Modalities Test (Smith, A. Los Angeles: Western Psychological Services, 1973) provided comparisons of efficiency in substituting numbers for nonverbal symbols in 90" intervals in two different language modalities, writing and speaking. SDMT studies of symbol processing in the development of reading in 497 kindergarten children (mean age 80 mos.) reported significant (r =.01) correlations between ability to substitute numbers for nonverbal symbols orally and reading skills developed 1 yr. later (Hutton, S.G. READING DIFFICULTY: Early and Economic Identification of Children at Risk. Ph.D. Dissertation, Univ. of Cape Town, 1973). SDMT tests of adult aphasics since 1967 afforded comparisons of ability to substitute numbers for nonverbal symbols in two different language modalities, writing and speaking. Tests of 126 aphasics with vascular (mean age 48 yrs.-duration aphasia 18 mos.) and 36 with traumatic (correspondingly, 28 yrs. 25 mos.) cerebral lesions also provided data for correlating efficiency in writing and in speaking SDMT responses with performance in tests of speech, comprehension, reading and writing (Schuell, H. Minnesota Test for Differential Diagnosis of Aphasia. Minn: Univ. of Minn. press, 1965). Correlations of written and oral SDMT scores showed a Pearson r =.79 for the stroke cases and .80 for the traumatic aphasics. Comparisons of groups differentiated into four different levels of written (0, 1-12,13-23,24-56) and then oral (0,1-9,10-19,20-56) SDMT scores showed systematically higher levels of residual speech, comprehension, reading and writing as levels of SDMT written or oral scores increased. Groups differentiated according to written or oral SDMT levels also revealed statistically significant differences in mean scores of all four language functions as well as in the alternate SDMT form. Although writing consistently showed the greatest impairment, in each group mean scores in tests of speech, comprehension, reading and writing were comparable, with no overlapping between groups. Despite handicaps in writing responses with the left nonpreferred hand, comparisons of hemiplegics and nonhemiplegics revealed several hemiplegics had higher written and oral SDMT scores and language functions were correspondingly higher than in nonhemiplegic aphasics equated for age, education, etc. Retests of 80 aphasics after intensive language therapy showed slight changes in nonverbal tests. However, Schuell tests showed significant improvement in all four language functions with corresponding increases in written and oral SDMT scores. The findings support definition of aphasia as "symbolic disorders resulting from impairment of some central integrative mechanism" (Darley, F.L. Diagnosis and Appraisal of Communication Disorders. Englewood, N.J: Prentice-Hall, 1964). The comparable degrees of impairment in all four language functions in each group differentiated for abilities to process symbols in written and oral SDMT tests - regardless of the presence or absence of hemiplegia - suggests that although associated motor and sensory defects may further depress specific expressive or receptive language functions, the apparent variations of aphasic syndromes are superficial superimpositions on the varying degrees of impairment in symbol processing that underlies all language functions.

616 A PARADIGM FOR POSITION LEARNING IN THE CRAYFISH CLAW. Carl E. Stafstrom* and George L. Gerstein. Depts. of Biophysics and Physiology, Sch. of Med., Univ. of PA, Philadelphia, PA 19174.

Horridge (1) has demonstrated a paradigm by which insects can learn leg position. We have examined whether a higher invertebrate could be similarly treated in order to creat a preparation useful for neurophysiological study of learning. The crayfish dactyl moves relative to the propodite in a single plane, is controlled by two muscles and five motor axons, and has been extensively studied (2). In our preparation, crayfish were restrained so that only the dactyls were allowed to move; a position-sensing potentiometer was attached by a thread to each dactyl. Two position thresholds (θ_p and θ_c) were defined for the "positional" and "control" claws respectively. Circuitry provided a train of shocks (1-5V, 10/sec) to the closer muscles whenever the positional claw's dactyl moved past θ_p. Thus the positional claw received shocks dependent on its position, while the control claw received shocks whenever the positional claw did, but irrespective of its own position. Each animal thus acted simultaneously as experiment and control.

Experiments were divided into three sections:

a. During the control period of 30 min., neither claw received shocks.

b. During the 25 minute training period, shocks were delivered to both claws whenever the positional claw opened past θ_p.

c. A test period of 60 minutes followed during which neither claw received shocks.

Positions of both dactyls were written out on an inkwriter; another trace was used to mark whether the positional claw had opened past θ_p. Crossings of θ_c were not monitored, but were later constructed from the inkwriter record. Using five minute epochs, the fraction of time spent by each dactyl above its respective θ was measured and plotted.

Results show a significant decrease in the fraction of time the positional claw is open, whereas there is no change in the control claw. Of 12 Procambarus clarkii specimens, six demonstrated this position learning, with left and right claws learning equally well. Four of the 12 learned the paradigm after two consecutive training periods (separated by one test period); two of the 12 failed to learn at all. Two of the four Cambarus diogenes animals showed position learning, one required two training periods, and one did not learn at all.

To control for the specific effects of the stimulus on claw musculature, other anatomical areas have been shocked instead. Preliminary results indicate that the claw learns position even when the tail or walking legs are shocked. This preparation therefore serves as a promising model for future neurophysiological probings into the cellular basis of learning. (Supported by NS 05606.)

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[2]C.A.G. Wiersma and R.T. Adams, Physiol. Comp. Oecol., 3: 20-33 (1950).

617 CHANGES IN HIPPOCAMPAL SYNAPTIC DENSITY WITH INCREASED LEARNING EXPERIENCES IN THE RAT. Richard Altschuler Department of Anatomy, University of Minnesota, Minneapolis, Minnesota 55455

A learning enriched environment has been shown to result in an increased synaptic density in the rat occipital cortex. (Möllgaard et al., Int. J. Neurosci. 2:113 1971). Area CA3 of the hippocampus, implicated in many learning hypotheses, was examined to determine if similar changes might occur.

Ten 27-day-old female rats were equally divided into two groups: controls were maintained in a normal laboratory environment with minimal stimulation; experimentals were housed in a learning enriched environment and given ten weeks of exposure to learning tasks. After eighty days the hippocampi of three animals from each group were prepared for electron microscopy. Thin sections were cut from area CA3 as determined by light microscopy, electron micrographs were taken from random areas and prints were coded. Synaptic density, the relative area occupied by synapses, was determined using point-counting quantitation; 1036 total points per print were counted. Synapses were defined as having a pre-synaptic accumulation of vesicles, a post-synaptic thickening and two distince membranes. The mean density of synapses in the experimental group was $1.97 \pm 0.5\%$ and that of the control group $1.04 \pm 0.3\%$ ($p = 0.001$). The large increase in the amount of synaptic area in the group exposed to a greater amount of learning experiences suggests that synaptic changes in the hippocampus may be associated with learning processes.

618 PREPROCESSING OF VOWELS WITH A SIMPLE NEURAL MODEL. James A. Anderson, Jack Silverstein*, and Stephen A. Ritz*
Divisions of Applied Mathematics and Biological and Medical Sciences and Department of Linguistics, Brown University, Providence,RI 02912

A model for memory, based on neurophysiological considerations, has been proposed previously. We assume that (1) nervous system activity is most usefully represented as the set of simultaneous individual neuron activities in a group of neurons, (2) different memory traces make use of the same synapses, and (3) synapses associate two patterns of neural activity by incrementing synaptic connectivity proportional to the product of pre- and post- synaptic activity (a Hebbian rule) forming a matrix of synaptic connectivities. We extend this model by (1) introducing positive feedback of a set of neurons onto itself and (2) allowing the individual neurons to saturate, that is, they cannot fire faster or slower than certain limits. Positive feedback tends to force the pattern of neural activity into stable corners of a high dimensionality hypercube. A hybrid model arises, partly analog and partly binary. The model has behavior reminiscent of 'categorical perception' in that large regions of initial neural activity will end in the same corner. We apply this model to the perception of vowels. We wish to demonstrate that this model can serve as an efficient pre-processer, which takes a noisy stimulus, a spoken vowel, and puts it into a noise-free standard form. For a test, we used acoustic representations of nine spoken Dutch vowels, taken from a paper by Klein, Plomp, and Pols (J. Acoust. Soc. Amer.,48, 999-1009 (1970)). We apply the model to it and show that after several thousand learning trials, input vowels, initially close together, are associated with separate stable corners.

619 MEMORY DOMAINS ESTABLISHED DURING LEARNING PROCESSES.** Photios A. Anninos and Silvio Zenone*. Dept. of Physics, Concordia University and Dept. of Physics, Dawson College, Montreal, P.Q.

Incoming stimuli, after having been transduced through our senses, activate certain parts of the brain. This activation produces directly a reinforcement of the coupling coefficient of the neurons involved. In addition, as a result of the learning processes, neurons outside the particular region, undergo also a reinforcement of their coupling coefficients. As a result of these processes the neuron's firing threshold decreases, slowly initially and then more drastically as the learning process progresses.

This variation in threshold has an interesting effect on the previously found hysteresis curves (J. of Theoret biol. 26, p.93 and p. 121, 1970): we have found, with our preliminary computer-simulation runs that the single hysteresis loops, obtained with a constant threshold, are replaced by multiple ones, analogous to the hysteresis domains of ferromagnetism. It could be possible to interpret the occurence of such domains as the representation of multiple mapping of the memory. In such representation if for any reason one of the hysteresis loop is destroyed, still the memory stored can be recalled.

**This research is being supported by NRC grant A9974.

620 ASSOCIATIVE STRENGTH AND SLOW POTENTIALS. Charles H.M. Beck. Dept. of Psychol., U. of Alberta, Edmonton, Alberta, Canada, T6G 2E9.

Wagner and Rescorla (In Boakes & Halliday, Inhibition & Learning, 1972) argued that the associative strength of one element of a compound stimulus grows toward the maximum associative strength as the aggregate associative strength of all cues present on that trial approach the maximum associative strength. Wagner and Rescorla suggest that there are similar dependencies between CS's in successive trials of two choice discriminations. The background stimuli common to the two stimuli serve as the joint compound. Thus increased rate of responding to CS1 following extinction of CS2 would be predicted in the behavior contrast (Reynolds, JEAB 4:57, 1961) paradigm. The Wagner Rescorla model would predict that the higher the initial associative strength of the CS2, the greater would be the positive contrast effect on CS1. Spence's (PR 54: 89, 1952) theory would predict that the greater the associative strength of the CS2, the more trials it would take to extinguish it and this greater inhibition would generalize to CS1 resulting in a greater depression of responding to it than to the CS1 coupled with the weak associative strength CS2. Thepresent experiment utilized monkeys in a successive reaction time discrimination to resolve this query. The results support the Spence model in that slowing of the speed of response to CS1 was the only significant effect observed. The results support the Wagner-Rescorla thesis in that the CS1 paired with the high associative strength CS2 was significantly less depressed than the response speed to the CS1 paired with the low associative strength stimulus. Correlated changes in transcortical slow potentials recorded while the monkeys performed the discrimination were observed.

621 CONTRASTING MEDIAL AND LATERAL SEPTAL UNIT RESPONSES DURING CLASSICAL CONDITIONING. Theodore W. Berger* and Richard F. Thompson. Dept. Psychobiol., UCI, Irvine, CA. 92717.

Unit activity was simultaneously recorded from the hippocampus and lateral septal nucleus during classical conditioning of the rabbit nictitating membrane response to tone CS. Lateral septal discharges showed essentially the same pattern of firing, both within and across trials, as found in the hippocampus--that is, large increases over spontaneous rates early in training, the increases temporally shifting into the CS period as behavioral conditioning develops. Definite differences between hippocampus and lateral septem were found with respect to rates of increase across trials and latencies of unit firing. Simultaneous recordings from hippocampus and medial septal nucleus revealed that the latter activity responded to stimulus parameters of the paradigm--tone and air puff (UCS) presentations. Medial septal responses had a tendency to habituate across trials. Unpaired controls showed both the hippocampal and lateral septal increases to be a result of conditioning only. In contrast, medial septal responses to tone and air puff were found in unpaired controls as well.

622 THE HUMAN MIND, THE EGO AND COMPUTER SOFTWARE. W.R.Blackmore Fac.Administrative Studies, York U. Downsview, Ontario.

Just as in a large computer system there must be a large program (called software) which resides in the machine (hardware) to supervise the whole installation and make the right components do the right things at the right nanosecond in executing the users' programs, so too must we have a mind.We have no idea of how we store or retrieve memories, form associations, or intuit another's feelings. We are not taught these skills, nor do we learn them. These abilities and many others simply emerge in due course. Thus Behaviourists have completely missed the boat in supposing that we are simple stimulus-response beings. Psychoanalysis has long agreed that there must be an ego, but erred in supposing this to be only a metaphorical structure. Arguments advanced here maintain that all animals with brains (particularly mammals) must have the equivalent of software. Animal software is embodied partly in living tissue (e.g. reticular system, Limbic system) and partly in hormonal control. It is obvious that we have no idea how we have ideas, dream, compose sentences, develop hypotheses, etc. We somehow are able to do these things when our software is ready. Thus we have the cognitive developmental stages described by Piaget and the sequence of moral development reported by Kohlberg. The fact that the identical developmental sequence emerges in all humans around the world demands the existence of an inherited hierarchical organization. Consciousness will also be discussed.

623 MEMORY DISRUPTION PRODUCED BY UNILATERAL, LOW INTENSITY, SINGLE PULSE STIMULATION OF THE AMYGDALA. Jeffrey D. Cross* and Irving J. Goodman* (SPON: J.L. Culberson). Depts. Psychology and Psychiatry and Behavioral Medicine, West Virginia University, Morgantown, W.Va. 26506.

There have been a variety of procedures used in attempting to assess those areas of the brain that appear to be involved in memory storage and retrieval. These procedures range from global disruption of brain activity to disrupting the activity of highly circumscribed areas. The present study attempted to limit both the volume of brain tissue disturbed and the duration of the disruptive stimulus by employing a single electrical pulse (100uA;0.5m sec) delivered unilaterally to the brain of rats via chronically implanted electrodes. Among the groups employed were animals stimulated in the amygdala, dorsal hippocampus, and several other forebrain structures. A number of control groups were also included. Using a step-down latency testing procedure, retention deficits for an aversive electric gridshock were demonstrated 24 and 48 hours after treatment by those animals that received unilateral amygdalar stimulation within 0.5 seconds after the gridshock. Similar unilateral stimulation of the dorsal hippocampus, caudate nucleus, and several other structures, on the other hand, did not result in such deficits. Retention deficits were reflected in maintained short step-down latencies on the 24 and 48 hour recall tests while retention of the aversive gridshock effects was inferred from significantly increased step-down latencies. In addition to the behavioral signs of recall loss, correspondingly different patterns of heart rate change during recall testing were noted between controls and amygdala stimulated subjects. The results suggest that information storage and retrieval may involve a bihemispheric system, and that improper functioning of the amygdaloid complex in either hemisphere may be sufficient to disrupt this system.

624 EFFECT OF TRAINING ON PYRIMIDINE NUCLEOTIDE METABOLISM IN MOUSE BRAIN. S. Diane Davis*, Daniel J. Entingh and Terri Damstra. Dept. Biochem. & Neurobiology Program, Univ. Nor. Carolina, Chapel Hill, NC 27514.

The effects of footshock-avoidance training and stimulation (yoking) upon the distribution of intracerebrally injected ^{14}C-uridine among RNA, pyrimidine nucleotides, and nucleotide sugars were studied in male C57Bl/6J mice. After 45 min of labeling and 15 min of training or yoked stimuli mice were killed by immersion in liquid N_2. Nucleotides and nucleotide sugars were extracted and RNA precipitated with acidic ethanol. Twelve radioactive pyrimidine nucleotides and nucleotide sugars were separated and identified by thin-layer chromatography.

The only significant effect of training was an increase in the amount of radioactivity recovered from the brains as ^{14}C-uridine; there was a relative decrease in radioactivity in RNA, nucleotides, and nucleotide sugars. No changes were observed in the brains of yoked mice. It is possible that the changes reflect altered energy utilization in the brains of learning mice.

625 EFFECTS OF VOMERONASAL-NERVE DISRUPTION ON AN ILLNESS-INDUCED TASTE AVERSION AND SHOCK-MOTIVATED COMPARTMENT AVOIDANCE. Ralph L. Elkins, Stephen H. Hobbs, and Gary Bohnert* VA Hospital, Augusta, Ga. 30904

Numerous experiments have studied the effects of aspiration lesions of the olfactory bulbs on a variety of behavioral phenomena. In addition to removing olfactory bulb tissue, such lesions probably destroy fibers of passage including vomeronasal fibers which arise from the vomeronasal (Jacobson's) organ and terminate at the accessory olfactory bulbs. Olfactory bulb aspirations approximating total bulbectomies in albino rats disrupt the acquisition both of an illness-induced taste aversion and shock-motivated compartment avoidance. In contrast, smaller lesions restricted to the anterior third of the bulbs disrupt taste-aversion conditioning without influencing the acquisition of compartment avoidance (Elkins & Hobbs, Society for Neuroscience, 1974). However, both lesions may have disrupted vomeronasal fibers of passage. A reexamination of taste-aversion acquisition and the conditioning of shock-motivated compartment avoidance by albino rats in which the vomeronasal fibers were interrupted anterior to the bulbs was undertaken. Efforts to sever vomeronasal fibers with knife cuts anterior to the cribriform plate produced excessive bleeding and a high mortality rate. This problem was eliminated through bilateral cauterization of the vomeronasal fibers. Postmortem examination revealed extensive damage to vomeronasal fibers and additional lesion verification is in progress. Subjects undergoing cauterization showed no deficiency in acquiring either a cyclophosphamide- (Cytoxan^{R}, Mead Johnson) induced taste aversion or shock-motivated compartment avoidance. Therefore it is unlikely that our previous behavioral results (Elkins & Hobbs, 1974) are attributable to interruption of vomeronasal fibers of passage at the level of the olfactory bulbs.

626 RETROGRADE AMNESIA IN CHICKS: INDUCTION BY L-PROLINE IS NOT ACCOMPANIED BY OCCULT SEIZURES. Lauren K. Gerbrandt*, Michael J. Eckardt*, Mark I. Simon*, and Arthur Cherkin (SPON: J. L. Davis). Dept. Psych., California State Univ., Northridge, CA 91324, and Psychobiology Research Laboratory, VA Hospital, Sepulveda, CA 91343.

Intracerebral injection of L-proline (PRO) induces retrograde amnesia (RA) in chicks (Cherkin, Eckardt and Gerbrandt, Science, in press, 1976). PRO may interfere with an initial step in memory formation by blocking the transformation of a patterned release of neuronal glutamate into a corresponding pattern of dendritic swelling (Van Harreveld and Fifkova, Exp. Neurol. 81, 455, 1976). Alternatively, PRO may cause occult seizures, as was found with puromycin-induced amnesia (Cohen and Barondes, Science 157, 333, 1967). We investigated the latter possibility. Chicks (N=25) were implanted with bilateral electrodes in the ectostriatum or posterior neostriatum. After a 15-min adaptation and EEG baseline period, chicks received intracerebral injections of 10 µl per hemisphere of 300mM PRO (N=15), or 300 mM L-isoleucine (N=5), or physiological saline (N=5). EEG was recorded for an additional 10 min after injection. None of the injections produced isoelectricity or seizure spiking in polygraph records. An offline spectral frequency analysis was used to compare the PRO group with the isoleucine and saline controls. At 1.5-3.0 min after PRO injection, the incidence of high frequency zero-crossing (above 8 Hz) was significantly increased, and low frequency activity (below 8 Hz) was significantly decreased selectively in the PRO-injected group. Integrated multiple unit and EEG activity were slightly and briefly depressed for 10-30 sec after PRO injections. The marginal electrophysiological effects of PRO seen here are not known to be sufficient to induce RA. Thus, it remains a viable suggestion that L-proline blocks memory formation in the chick via a morphological expansion of dendritic spines.

627 PASSIVE AVOIDANCE LEARNING IS INHIBITED BY ANTISERUM TO BRAIN GANGLIOSIDES. Stephen E. Karpiak, Liselotte Graf* and Maurice M. Rapport, Division of Neuroscience, New York State Psychiatric Institute and Departments of Biochemistry and Pathology, Columbia University, College of Physicians and Surgeons, New York, N. Y. 10032.

In previous studies we have found that one trial, step-through passive avoidance learning is inhibited by intraventricular injection of antisera against synaptic membranes or S-100 protein, whereas it is not inhibited by antisera to 14-3-2 protein, galactocerebroside, or myelin. Since antisera to synaptic membranes contain antibodies reacting with gangliosides, and since gangliosides are important components of synaptic membranes, we have studied the effect of antiserum to gangliosides on this behavioral paradigm. Antisera prepared against total gangliosides of bovine brain (Pascal et al., 1966) are known to react with G_{M1} and G_{D1b} ganglioside species but not with G_{D1a} and G_{T1} gangliosides (Rapport and Graf, 1969). Rats were injected intraventricularly immediately after training with 20 µl of native antiserum or of antiserum from which the antiganglioside antibodies had been completely absorbed with pure G_{M1} ganglioside. They were retested on the step-through passive avoidance task 7 days later. In rats receiving the antiganglioside serum almost 95% inhibition of learning was seen whereas rats receiving absorbed antiserum were not inhibited. We have previously shown that these antiganglioside sera cause epileptiform spiking in the EEG (Karpiak et al., 1976). We conclude that antisera to brain gangliosides are effective in interfering with synaptic connections that are involved in both behavioral and electrophysiological events.

628 SUCKLING MOTIVATES INSTRUMENTAL LEARNING IN PREWEANLING RATS. John T. Kenny* and Elliott M. Blass. Dept. Psychology, Johns Hopkins University, Baltimore, Md. 21218.

To determine whether neonatal rats could learn an appetitive task and whether suckling is a motivated act, rats 7, 12, 17 and 21 days old were maternally deprived for 24 hr. and trained in a Y maze. Each correct response was rewarded by allowing the rat to suckle a non-lactating nipple of their anesthetized mother for 30 sec. After attaining criterion (8 of 10 correct responses), reversal training was instituted. Two days later reversal retention was assessed. Rats at all ages tested learned, reversed and retained the discrimination. There was no improvement with age. Thus, suckling, like adult ingestive behavior, is a motivated and not a reflexive act. Moreover, even 7 day old rats, whose cortical development is exceedingly primitive, learn, reverse and retain a simple spatial discrimination. Implications for the neurology and neurochemistry of learning, memory and motivation will be discussed. Supported by Grants in aid of research BMS 75-01460 and AM 18560.

629 TIME-DEPENDENT DISRUPTION OF MORPHINE TOLERANCE BY ELECTROCONVULSIVE SHOCK AND DISCRETE BRAIN STIMULATION. Raymond P. Kesner. Dept. Psychol. Univ. of Utah, Salt Lake City 84112.

In order to investigate the possibility that tolerance to morphine may represent a form of learning, which may be mediated by memory processes and cellular mechanisms similar to those underlying conventional learning, rats were given electroconvulsive shock (ECS) or discrete brain stimulation [frontal cortex, caudate or periaqueductal gray (PG)] 5 min or 3 hrs after an initial injection of 30 mg/kg dose of morphine sulfate. Forty-eight hours later rats received a second injection of morphine (15 mg/kg) followed by an assessment of pain threshold to electric shocks. Compared to appropriate controls, ECS, frontal cortex, or PG stimulation produced a disruption in development of 1-trial tolerance to the analgesic effects of morphine, but only when applied 5 min not 3 hrs after initial administration of morphine. Caudate stimulation had no disruptive effects. In the case of PG stimulation the time-dependent disruption was superimposed upon an electrode-induced lesion, which disrupted the development of tolerance to morphine without altering the pain threshold to shocks in absence of morphine. It is suggested that development of tolerance may be mediated by cellular mechanisms and memory processes similar to those thought to underlie conventional learning.

630 CHANGES IN EVOKED POTENTIALS DURING CONDITIONING. Zaven S. Khachaturian, Tsung-Ming Shih and Kurt L. Reisler*. Dept. Psychiat., Sch. Med., Univ. Pgh., Pittsburgh, PA. 15261.

There is considerable evidence that some of the reported changes in evoked potential (EP) amplitude and component during conditioning may be attributed to non-specific processes while other similar changes may be due to associative processes. To distinguish more specifically the relative influence of these two processes on EPs, ten chronically implanted cats were studied in a flaxidelized preparation. During three phases (habituation, conditioning and extinction) of the experiment the animals were concurrently presented a relevant (CS) and irrelevant (IS) stimuli. The CS consisted of four light flashes or clicks delivered at the rate of 1/2 sec. The IS was delivered at the same rate as the CS but was continuously present. The CS and IS were always out of phase by one second and were of different modalities. The unconditioned stimulus was a mild shock delivered 500 msec. following the CS. The results indicated three different types of changes in the averaged EPs elicited by the CS and IS during conditioning: a) Amplitude increases and component changes in CS-EP alone; b) Amplitude changes in IS-EP alone and c) Similar changes in both CS and IS EPs. The first two types of changes generally were observed in polysensory structures such as the pulvinar and mesencephalic reticular formation. The nonspecific type of changes (C) were generally observed in the primary sensory pathways such as lateral geniculate nucleus and medial geniculate nucleus. These results suggest that in evaluating changes in EPs during conditioning more careful consideration must be given to both specific (i.e., changes limited to CS-EPs or IS-EPs) and nonspecific changes (i.e., similar changes in both CS- and IS-EPs) in electrical activities at each brain locus studied.
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631 RETENTION DEFICITS FOLLOWING POST-TRIAL DOPAMINE INJECTION IN RAT NEOSTRIATUM. Haing-Ja Kim* and Aryeh Routtenberg (SPON: Barbara Jones), Cresap Neuroscience Lab., Northwestern Univ., Evanston, Ill., 60201.

Post-trial manipulation of neurons in substantia nigra (SN) either by electrical stimulation (Routtenberg and Holzman, Sci. 181 (1973) 83) or intranigral injection of picrotoxin (Kim and Routtenberg, Brain Research (1976) in press) disrupts 24-hr retention of step-down passive avoidance. Since the neostriatum, one nigral projection field, has been implicated in the memory consolidation (Wyers, et al. Exp. Neurol. 22 (1968) 350), activation of the nigroneostriatal dopaminergic pathway might have played a role in the observed retention deficit. We have, therefore, examined the effect of unilateral dopamine (DA) injection (5 μg/1 μl in 2 min) into neostriatum given 5 min after learning on retention studied 24 hr later. In order to prevent rapid degradation of injected DA by monoamine oxidase (MAO), i.p. injection of an inhibitor, nialamide (15 mg/kg), was given 1 hr prior to intrastriatal DA injection. Since preliminary experimentation showed that intrastriatal injection of ascorbic acid (0.2 μg/μl), an antioxidant, caused a retention deficit, ascorbic acid was not added to the DA solution. Intrastriatal DA injection in combination with i.p. nialamide produced a significant retention deficit relative to intrastriatal saline-injected controls pretreated with i.p. nialamide. Nialamide i.p. injection alone had no effect on retention of either unoperated or implanted control animals. These results call attention to a potential role for DA in neostriatum in retention of passive avoidance. Since injection of DA was given 5 min after learning, the retention disruptive effects observed were unlikely caused by impaired sensory processing or reduced strength of learning, but were more likely related to post-learning consolidation processes. Supported by The A. P. Sloan Foundation, NS 10768, MH 25281 and BMS 19481 to A.R.

632 MODULATION OF AUDITORY CORTEX UNIT RESPONSIVENESS DURING PERFORMANCE OF A CLASSICAL CONDITIONING TASK. Leonard M. Kitzes*, Glenn R. Farley*, and Arnold Starr, (SPON: M. Demetrescu). Depts. of Medicine (Neurology) and Psychobiology, University of California, Irvine, Irvine, CA. 92717.

We are studying the response properties of single auditory cortex units in muscle relaxed, unanesthetized, pre-trained cats performing a classical pupillary dilation conditioning task. The conditioned stimulus is a 500 msec. duration white noise burst and the unconditioned stimulus, occurring 5 seconds later, is a train of shocks to the tail. Best frequency, 100 msec. duration tone bursts presented continuously at 1 second intervals throughout the set of conditioning trails are used to probe for changes in single unit responsiveness occurring as a function of the temporal relationship of the tone to the behavioral conditioning trails. The great majority of units (20 of 25) exhibited modified responses to the behaviorally irrelevant probe tones or altered spontaneous discharge rates during and following a conditioning trial. Response modification may take the form of an increase or a decrease of driven or spontaneous discharge rates and frequently involves modulation of both types of activity. Of the 5 cells that showed no modulation following CS presentation, 1 exhibited augmented driven and spontaneous discharge rates following presentation of shock. Although presentation of a 500 msec. white noise burst to naive cat will sometime (7 of 27) alter responses to subsequently presented probe tone, our data indicates that the modulation is not due to an intra-modality interaction between the white noise and probe stimuli. Whether the modulation is a function of associative or generalized variables such as, arousal level cannot be answered at this time. However, these results indicate that responses of single auditory cortex unit can be crucially affected by the behavioral context in which acoustic stimuli occur.

633 EFFECTS OF CLOSED HEAD INJURY ON STORAGE AND RETRIEVAL IN MEMORY AND LEARNING OF ADOLESCENTS. Harvey S. Levin and Robert G. Grossman. Div. Neurosurg., Univ. Texas Med. Branch, Galveston, Tx. 77550.

Disruption of memory following closed head injury (CHI) in adults has been the subject of numerous studies, whereas few investigators have considered this problem in children and adolescents. The selective reminding procedure of Buschke and Fuld (Neurology 24:1019, 1974) was employed to assess short term recall, long term storage and retrieval of a word list by adolescents who had sustained CHI of varying severity. In comparison with a control group matched for age and sex, head injured subjects demonstrated impairment of both storage and retrieval of test items which were presented serially following recall failure. Normal adolescents progressively incremented their long term storage and retrieval across trials; head injured patients were more dependent upon short term recall of words, necessitating repeated presentations. Upon entering words into storage, retrieval by head injured patients was uncertain. When analysis was confined to the proportion of long term retrieval that was consistent, this value was found to be significantly greater in control adolescents. The proportion of consistent long term retrieval was inversely related to duration of coma in the head injured group. Neurological findings were considered in relation to performance. Clinical techniques for assessment of memory and remediation were discussed.

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634 EFFECTS OF CYCLOHEXIMIDE ON LEARNING AND MEMORY IN THE COCKROACH. Kathryn L. Lovell. Biophysics Dept., Mich. State Univ., E. Lansing, MI 48824

Cycloheximide (CXM), a protein synthesis inhibitor, produces memory deficits in vertebrates when administered before training, but the drug does not impair initial acquisition in vertebrates. However, previous experiments on headless cockroaches trained to lift a leg to avoid shock indicated that CXM impaired acquisition, but had no observed effect on retention. The research described here investigates this apparent phylogenetic difference in the effects of CXM on learning and memory processes. Experiments were designed to determine if the CXM-induced acquisition impairment is a general phenomenon in cockroach learning. CXM was injected before training into cockroaches trained under one of three conditions: (1) headless cockroaches trained to lift a leg to avoid shock; (2) intact cockroaches trained to lift a leg to avoid shock; (3) intact cockroaches trained to turn left or right in a T-maze to avoid shock. The results confirmed that CXM prolongs the time required for headless animals to reach a given criterion of leg lift learning and demonstrated that CXM has no impairing effect on leg lift or T-maze learning in intact cockroaches. Thus the CXM impairment of acquisition is specific to the headless preparation.

The effects of CXM on retention were investigated in cockroaches trained to turn left or right in a T-maze. CXM was injected before training, and retention was tested at intervals up to 22 hours after training. A dose of CXM which inhibited protein synthesis in the nervous system by over 90% did not affect learning or produce retention deficits at any interval, although the drug increased the activity of the animals in the maze.

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635 COLD WATER IMMERSION: INFLUENCE ON RECALL. Sheilagh Martin*, K.E. Cooper and R. Sainsbury*. Division of Medical Physiology and Department of Psychology, University of Calgary, Calgary, Alberta, T2N 1N4.

Long term cold water immersion can produce irrationality, delirium and unconsciousness. No thorough study has been made in humans of the psychological effects (e.g. memory deficit) which might accrue as a result of short term immersion.

Experiments were carried out to investigate the effects of cold water immersion on memory. In Protocol I, 13 subjects were shown a stimulus set of 25 slides, each exposed for 2 sec, before immersion in cold water (15.0° C) for 4 min. Recall tests were administered following exit from the water. Protocol II involved 11 subjects who were shown slides 1 min after immersion in the cold water and subsequently tested as in Protocol I. These experiments were repeated in warm water (33.0°C). There was a significant difference ($p < 0.05$) in the number of errors during the recall tests between the warm and cold water conditions as well as between the control and cold water in Protocol II. A rank correlation test of the number of errors and the mean % change in end-tidal pCO_2 in the cold water showed statistical significance ($p < 0.001$). No significant difference was noted in Protocol I.

These results do not appear to be influenced by offering monetary incentives. The data support the possibility of a recall deficit as a result of short term cold water immersion. The area of difficulty may lie with either information acquisition or processing and an inability to recall due to changes in cerebral blood flow.

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636 RETROGRADE AMNESIA INDUCED BY STIMULATION OF THE ENTORHINAL CORTEX. Joe L. Martinez, Jr., James L. McGaugh, Joe S. Lacob* and Carolyn L. Hanes*. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717

Many studies have investigated the role of hippocampal stimulation on memory, but few have investigated the effects of stimulating one of its major afferents, the entorhinal cortex. In this study the effect of two levels of stimulation (300 uA & 3 mA) given 60s or 3h after training in a one-trial, water-motivated, inhibitory (passive) avoidance task was investigated. One footshock level (2 mA for 2s) was used. The animals were given 6 days of pretraining, 1 training trial and tested 24h later. The rats were bilaterally implanted in entorhinal cortex with chronic indwelling twisted bipolar electrodes; additionally, cortical screw electrodes were placed epidurally over the frontal and posterior cortex. EEGs were recorded before and after the stimulation.

The results showed that the stimulation with 3 mA, but not 300 uA, 60s after training produced retrograde amnesia. However, if the stimulation was given 3h after training, 300 uA produced a significant amnesia, but 3 mA had no effect. Analysis of the EEG data indicated that cortical afterdischarges were seen in the 300 uA group 50% of the time and in the 3 mA group 75% of the time. Convulsions were observed in 90% of the animals given 3 mA, and were rarely seen in the animals given 300 uA. None of these effects were consistently related to the amnesia.

These findings indicate that the entorhinal cortex may play an important role in memory consolidation. The fact that a strong 3 mA stimulation was ineffective at 3h may indicate it is more like an electroconvulsive shock which has a short gradient of effectiveness, whereas a more localized 300 uA stimulation might have specific hippocampal amnestic effects.

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637 EFFECTS OF CONDITIONED STIMULUS INTENSITY ON CLASSICAL CONDITIONING AND SENSITIZATION IN THE SPINAL CAT. K.E. Misulis* and R.G. Durkovic, Dept. Physiol., Upstate Med. Ctr., Syracuse, N.Y. 13210.

Acute anemic decapitate cats were made spinal by a T10 cord transection. Conditioned and unconditioned responses were flexion reflexes measured by a force transducer attached to the tendon of the tibialis anterior muscle of a rigidly fixed hind limb. The CS was electrical stimulation of the (cutaneous) saphenous nerve of the same leg at 10 pulses per sec for 1.5 sec. Intertrial (CS) interval was 1 min. The US was electrical stimulation of the (cutaneous) superficial peroneal nerve at 30 PPS for 0.5 sec at an intensity which excited Aα and Aδ cutaneous fibers maximally. In the conditioning groups the US overlapped the last 0.5 sec of the CS. In sensitization controls the US followed the CS by 30 sec. Cats were allocated into six groups. Groups 1A and 1B: CS intensity was sufficient to excite a majority of Aα fibers, but was subthreshold for Aδ fibers. Groups 2A and 2B: The CS maximally excited Aα and Aδ fibers but the intensity was subthreshold for C fibers. Groups 3A and 3B: The CS maximally excited Aα, Aδ, and C fibers. Groups 1A, 2A, and 3A were presented with the classical conditioning paradigm. Groups 1B, 2B, and 3B were the corresponding sensitization controls. Results from conditioning groups (A) were compared with respective sensitization groups (B) to assess the magnitude of conditioned facilitation of the reflex. Statistical analyses showed that group 2A exhibited a significantly greater augmentation of response to the CS than did group 2B. No significant difference between conditioning and sensitization results were seen for groups 1 or 3. These data are consistent with classical conditioning studies discussed by Razran (1957) which show an inverted U-shaped function curve with increasing CS intensity. Supported by National Science Foundation Grant BNS 75-16747.

638 MASS ACTION RE-EXAMINED: SELECTIVE MODIFICATION OF SINGLE ELEMENTS WITHIN A SMALL POPULATION. Frank Morrell, Thomas J. Hoeppner* and Leyla de Toledo*. Dept. Neurology, Rush-Presbyterian-St. Luke's Med. Center, Chicago, Il. 60612.

Extracellular microelectrodes were used to simultaneously record single and multiple unit activity and local field potentials in the parastriate cortex of unanesthetized, paralyzed cats and rabbits. Receptive fields, preferred stimulus configuration and orientation specificity were determined for each cell or cell group. It was usually possible to define two stimulus configurations to which distinct responses occurred. One configuration was then paired with cutaneous shock in a Pavlovian conditioning paradigm; the other served as the differential (unreinforced) stimulus. Amplitude window discriminators were used to separate single units from the multiple unit record. Computed PST histograms of spike discharge were compared with averaged evoked potentials at each stage of conditioning. Conditional alteration of evoked potentials was frequently associated with concurrent modification of unit activity in some elements of the population and not others. Conditional alteration of unit activity occurred in the absence of evoked potential changes and was more specific and selective. Unit modification was usually differential with respect to reinforcement contingency and often different in pattern to the unreinforced stimulus. The evidence provides little support for the notion of coherent changes in large neuronal populations as a manifestation of plasticity and supports the selective involvement of specific cell populations in different aspects of the learning process.

639 CONDITIONING OF ARTERIAL BLOOD PRESSURE RESPONSES IN CATS WITH LESIONS OF THE NUCLEUS TRACTUS SOLITARII. M.A. Nathan, L.W. Tucker* and D.J. Reis. Lab. Neurobiol., Dept. Neurol., Cornell Univ. Med. Coll., New York, 10021.

Bilateral electrolytic lesions of the region of the nucleus tractus solitarii (NTS) in cats produced labile arterial hypertension (Circulation, suppl. III, p. III-61, 1974). The hypertension developed within hours after placement of the lesions and termination of the anesthetic. It consisted of increases in arterial pressure which lasted for several seconds to minutes. The labile hypertension was probably due to central disruption of the baroreceptor reflexes by the lesions. We now report the effects of classical conditioning of arterial pressure in cats with NTS lesions. The use of classical conditioning is the first step in a procedure designed to produce an animal model of fixed, neurogenic hypertension. The conditioning procedure involved presenting two tones of different frequencies for 10-30 sec. One tone was immediately followed by the delivery of an electrical shock and the other tone was never followed by the shock. We found that, in comparison to controls, the cats with NTS lesions showed: (1) the appearance of conditioned pressure responses after presentation of fewer tones (50-100), (2) larger conditioned pressure responses (120-200 mm Hg), and (3) more sustained responses (10-30 sec). We conclude that sympathetic activity increased by the classical conditioning procedure was unopposed by the inhibitory effects of the baroreceptor reflexes and thus resulted in the relatively rapid appearance of large and long lasting conditioned pressure responses.

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640 ULTRASTRUCTURE OF AN HABITUATING NEUROMUSCULAR JUNCTION. Nicki Ann Newby. Dept. Biol. Sci., Stanford Univ., Stanford, CA. 94305

The crayfish motor giant to fast abdominal flexor neuromuscular junction displays synaptic despression to rates of stimulation as low as one per minute (Bruner & Kennedy, 1970, Science 169: 92). This low frequency depression shares important parametric features with behavioral habituation. The fast flexor muscles are also innervated by non-giant excitatory motorneurons which do not exhibit low-frequency depression. The fact that the same postsynaptic muscle cells receive both types of innervation provided an ideal opportunity to investigate ultrastructural differences between habituating and non-habituating synapses. Each type of junction was identified by the use of electron dense dye (procion brown or procion rubine) which was selectively injected into the axon of each type of motorneuron in different preparations. It was found that both types of junctions contained slightly oval vesicles, approximately 500-600 Å in diameter, which were identical in size and shape for the two types. The motor-giant neuromuscular synapses had asymmetrically staining junctional membranes, with the presynaptic membrane staining less darkly than the postsynaptic. By contrast, the non-giant neuromuscular synapses had symmetrically staining junctional membranes. The cleft width for both types of synapses was similar (about 250-300 Å) and both occasionally contained dense cleft material. Thus, in this preparation, the most distinguishing feature of habituating synapses so far observed is asymmetry of the junctional complex.

641 INHIBITION OF SPINAL INTERNEURONAL ACTIVITY: A POSSIBLE NEURONAL SUBSTRATE OF HABITUATION. J.A. Pearson* and J.F. MacDonald* (SPON: D.P. Cain). Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

In previous reports of experiments carried out on acute spinal cats Groves and Thompson have described the characteristics of spinal interneurones whose changing responsiveness were considered to be responsible for habituation and sensitization of the flexor reflex. They were unable to provide any evidence to support the view that habituation might be a consequence of a progressive build up of inhibition.

In the present investigation the responses of interneurones in intact rat spinal cords to repetitive electrical cutaneous stimulation were studied. Several types of neurone were encountered whose activity changed as a consequence of repetition of the stimulus. The type which was of particular interest was spontaneously active and was inhibited by the stimulus. The period of inhibition progressively lengthened with each successive trial. The rate of build up of the inhibition was directly related to both the frequency and intensity of stimulation. With a stimulus intensity of 20mA and interstimulus interval of 1.5 sec, units were usually completely inhibited after 10-15 stimuli and activity often did not return for several minutes. Intravenous administration of strychnine prevented this build up of inhibition. Experiments have also been carried out on spinal rats. Numerous cells were encountered which were inhibited by cutaneous stimulation but in no instance did the inhibition show a progressive increase. It is tentatively concluded that habituation of the flexor reflex in the intact, but not spinal, rat may be partially a consequence of a build up of inhibition of spinal interneurones.

642 CENTRAL ACTIVATION AND MEMORY RETRIEVAL: FACILITATIVE EFFECT OF PRETEST INJECTION OF STRYCHNINE ON PASSIVE AVOIDANCE BEHAVIOR. Susan J. Sara and Jean-François Remacle°. Ctr.Exp.Comp.Psych., U. Louvain, 3041 Pellenberg, Belgium.

Performance of a passive avoidance response (PA) 24 h after training was assessed in rats treated with stychnine sulphate before retention test. In Exp I, one of the groups had been submitted to electroconvulsive shock (ECS) after training; in Exp II the training conditions were such that control animals had a poor 24 h test performance. In Exp I, three doses were used--0.1 mg/K,0.25 mg/k and 1.0 mg/K-- each dose was found to be active when compared to saline controls. 1mg/k was the most effective since it was the only dose at which there was no difference between ECS and non ECS groups. In Exp II only the 1 mg/k dose was used; in both experiments, with and without the amnestic agent, strychnine treated animals performed better than control groups. The drug had no effect on spontaneous step through latencies, activity in the experimental situation or on open field behavior at any of the doses investigated. The results were interpreted as evidence that this compound facilitated memory retrieval processes, thus underlining the fact that these processes are labile not only after a deficit due to ECS, but in under trained normal animals as well. Altering the functional state of the nervous system or simply lengthening the duration of the retrieval test (Sara, et al JCPP, 89:5, 1975) can effectively improve retrieval processes.

643 OPERANT CONDITIONING OF CORTICAL NEURONS WITH A TRACKING TASK
E. M. Schmidt, J. S. McIntosh, and M. J. Bak, Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

Previous experiments have shown that firing patterns of cortical neurons can be operantly conditioned. The objective of the current experiment was to determine, using operant conditioning techniques, the accuracy and speed of response that could be achieved by monkeys in controlling the firing frequency of single or multiple neurons. Intracortical microelectrodes were chronically implanted in the hand-arm area of the precentral motor cortex of 3 monkeys (Macaca mulatta). For each cortical neuron studied, the range of firing frequency from zero to near maximum was divided into eight equal zones, each indicated by a separate cursor light A second set of eight target lights indicated the firing frequency range required for a liquid reward. Sequences of 40 or 80 random target lights were presented to the monkey to quantify cell conditioning. Typical reward requirements were such that the firing rate of the contingent neuron be within the target zone for 1 second. If this condition was not met within 8 seconds a failure was registered and a new target light was presented. Neurons whose firing was associated with specific movements were easily conditioned while neurons that were unrelated to movements were difficult or impossible to condition. Our neuronal conditioning paradigm appears to condition specific movements that the monkey refines as he learns the task. The best performance achieved in controlling neuronal firing rate resulted in an average response time of .99 seconds from target presentation to initial target acquisition. The range of firing frequency and speed of response may be useful as a control signal for prosthetics if stable long term recording can be achieved.

644 IMMUNOLOGICAL STUDIES OF SPECIFIC GOLDFISH BRAIN PROTEINS WHOSE METABOLISM INCREASES AS A RESULT OF TRAINING. Victor E. Shashoua. Biological Res. Lab. McLean Hosp., Dept. Biol. Chem., Harvard Med. Sch., Belmont, MA 02178.

The labelling pattern of three proteins (α, β and γ) in goldfish brain was found to change after the animals successfully acquired a new pattern of behavior (Brain Research (1976) in press). These proteins have now been isolated from the brain cytoplasmic fraction, purified by successive gel electrophoresis and used as antigens to immunize rabbits. Antisera containing antibodies to two of the proteins (β and γ) were obtained. These gave single precipitin bands when plated against the antigens and a mixture of the total cytoplasmic proteins. The distribution of β and γ in brain subcellular fractions and in a variety of goldfish tissues was determined by immunodiffusion methods. γ was specific to brain. The β protein cross-reacted but was not identical to a widely distributed substance in plasma, liver and kidney. Both β and γ appear to be species specific in that no cross-reactivity was obtained with mouse, chick or rat brain proteins. Immunological methods, in combination with double labelling experiments, were used to establish that the β and γ antigens were proteins which were normally present in goldfish brain. Both the β and γ antisera were equally capable of specifically precipitating the proteins which were differentially labelled after training as well as purified proteins of the same molecular weight present in the brains of control animals. These results suggest that the acquisition of a new pattern of behavior can increase the demand for specific proteins (β and γ) normally present in goldfish brain. (Supported by NINCDS grant number NS 09407.)

645 NEW LEARNING CAPACITY AND REMOTE MEMORY IN CHRONIC ANTEROGRADE AMNESIA. Larry R. Squire and Pamela C. Slater*. VA Hosp., San Diego, CA. 92161 and Dept. Psychiatry, UCSD, Sch. Med., La Jolla, CA. 92037.

Tests of new learning capacity and remote memory were given to a chronic amnesic patient who sustained a stab wound to the basal brain in 1960. Memory tests specifically sensitive to left or right hemispheric dysfunction indicated that the deficit was more pronounced for verbal material than for nonverbal material, in agreement with previous findings with this patient obtained 12 years previously (Teuber, Milner, and Vaughan, Neuropsychologia, 1968, 6, 267-282). Tests of remote memory that assessed memory for events from specific time periods (1950 to 1974) demonstrated a marked deficiency in memory for events occurring since 1960, together with a normal ability to remember events prior to that time. Cueing procedures improved memory for events since 1960 somewhat in this amnesic patient and in control subjects, but the marked difference between patient and control scores was maintained. Thus, cueing procedures did not eliminate the anterograde amnesia. The results suggest that the chronic amnesic syndrome described here is attributable to a deficit in storage of memory rather than to a failure of retrieval.

646 CORTICAL STEADY POTENTIAL (SP) SHIFTS AND RAPID LEARNING BY MONKEYS. John S. Stamm, Oliver A. Gillespie,* Steven C. Rosen and Barry B. Sandrew* Department of Psychology, State University of New York at Stony Brook, New York 11794

Monkeys, with nonpolarizable electrodes implanted in prefrontal, precentral and occipital cortex and across the eyes, were trained on delayed response (DR) and delayed matching-to-sample (DMS) tasks. The start of the trial (1-sec cue presentation) was contingent upon on-line computer detection of specified events for each group; namely: FSP - surface negative SP shift (50-100 μV, 2.5 sec.) from left prefrontal cortex (principal sulcus); MSP - SP shift of the same parameters from left precentral cortex; LEM - eye deviation to the right of 40°; and YC - controls, tested with intertrial intervals (ITIs) yoked to experimental monkeys. The median sessions (and means of total errors) to 90% correct performance on 12-sec. DR were: FSP - 6.0 (62.25); MSP - 11.5 (198.5); LEM - 13.0 (313.5); YC - 19.0 (328.6). When tested under off-line conditions with constant ITIs, all groups performed well, with transfer scores near 90%. During on-line testing with brief cues (100m sec.) only the FSP monkeys continued to respond at 90% correct performance. For testing on DMS there were no appreciable group differences in acquisition scores. The averaged ECGs showed the required pre-trial contingencies. The large FSP prefrontal shifts were not accompanied by similar events from right prefrontal, or other electrode locations. The ECGs from these electrodes remained essentially at baseline levels until cue onset, as did the recordings from the LEM and YC monkeys. The impressive rapid learning by the FSP monkeys appears to be the consequences of heightened attentive states that are reflected by the pre-trial SP shifts. (Supported by NSF Grant GB 35735-X1)

647 THE EFFECT OF STIMULUS INTENSITY IN A COMPUTER-SIMULATED MODEL OF HABITUATION J. C. Stanley* and S. I. Amari* (Spon. M. A. Arbib). Dept. of Comp. Sci., Univ. of Mass., Amherst, Mass., 01002.

One of the most intriguing aspects of habituation is the way the decrement varies with stimulus intensity. In some systems, relative habituation--defined as the decrement divided by the unhabituated response level--varies inversely with stimulus strength, while in others it appears to be independent of intensity. Response decrement in a given system will be determined by the characteristics of the plastic synapses and by the properties of the cells with which those synapses make contact. In order to explore the ways that cell thresholds can affect the form of response decrement, we have considered habituation in a simple model circuit.

In this model system, a bundle of afferent fibers makes contact at random with a rank of neurons. An external input activates a number of fibers proportional to the stimulus intensity. This activation results in a distribution of EPSPs in the cell rank. Each cell has a fixed threshold assumed to be a random variable chosen according to a given probability distribution. The cell fires if its total EPSP exceeds its threshold; the firing rate is the difference between the EPSP and the threshold. Formulas are derived giving the probability of cell firing and the average output rate as functions of stimulus strength and synaptic efficacy. Synaptic efficacy decreases during habituation training. This change is expressed in terms of a simple differential equation that captures the characteristics of exponential decrease and spontaneous recovery. Analysis and computer simulation indicate that with different forms of the equation for synaptic change, the average output rate of the cell rank can display relative habituation that either depends inversely on stimulus intensity or is approximately intensity-independent.

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648 POST-TRAINING INJECTIONS OF α-METHYLTYROSINE ALTER THE NORMAL OSCILLATION OF RETENTION PERFORMANCE IN RATS. Walter N. Tapp* and Frank A. Holloway (SPON: A. R. Zeiner). Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, Okla. 73190

Alterations in the rhythmic fluctuation of retention performance in rats were studied after the administration of α-methyl-p-tyrosine (αMT), a catecholamine synthesis inhibitor. One hundred and ninety-two rats were trained in a one-way, step-up active avoidance task at one of four times: 03.00-06.00; 09.00-12.00; 15.00-18.00; 21.00-24.00. αMT (50 mg/kg, i.p.) or an equivalent volume of saline was administered immediately after training. Retention performance was tested at one of four training-testing intervals. Retention performance of the saline groups was good at 12 hr and 24 hr but poor at 6 hr and 18 hr. These results replicate prior findings in normal rats across a variety of tasks. The rhythmic fluctuation of retention performance was altered after αMT. The 18 hr deficit was attenuated or abolished by αMT at all four training times. However, αMT abolished the 6 hr deficit only in the group trained at 21.00-24.00, or just after dark onset. αMT had little or no effects on 6 hr groups at other training times or on 12 hr and 24 hr groups. Prior studies in our lab have shown that lesions of the suprachiasmatic nucleus selectively abolished the 18 hr retention deficit. The present data also suggest the separation of the normal oscillation in retention performance into at least two periodic processes, in that the 6- and 18-hr retention deficits were differentially affected by pharmacological manipulation of catecholamines. The phase dependent effect of αMT on the 6 hr deficit may reflect the interaction of these two hypothetical processes.

649 CAUDATE LESIONS INCREASE NONREINFORCED OPERANT RESPONDING IN RATS. Robert L. Thompson and Robert C. Leslie, Jr.* Depts. Psychol., Hunter College, CUNY, New York, NY 10021, and State University College, Oneonta, NY 13820.

The character of behavioral changes in caudate-lesioned animals remains vexing. Among the processes appealed to are response perseveration, failures of inhibition, difficulty with proprioceptive information processing, and difficulty in shifting from one response mode to another. Our strategy has been to seek a chronic behavioral alteration in a steady state operant performance where interpretation and experimental analysis may be made with reference to basic concepts in conditioning theory. Hooded rats performed stably on a mixed schedule of continuous reinforcement (CRF) and differential reinforcement of "other" behavior (DRO). In the mixed schedule, trial opportunities for reinforcement and trials of nonreinforcement alternate randomly without differential exteroceptive cues. The normal animal detects when his own behavior is not "paying off" and stops responding until a new trial is signalled. Five rats surviving small bilateral lesions of the anterior caudate nuclei were compared to eight normal controls and seven rats with posterior caudate lesions. Anterior caudate lesions produced significantly more "runs" of nonreinforced responses, more responses per "run", and longer latencies to respond initially. Similar results were reported for monkeys (Thompson, 1963).

650 TRIGEMINAL INVOLVEMENT IN BRAINSTEM SELF-STIMULATION AND STIMULATION-BOUND BEHAVIOR. D. van der Kooy and A.G. Phillips. Dept. of Psychology, University of British Columbia, Vancouver, Canada, V6T 1W5.

The activation of norepinphrine-containing cell bodies in the locus coeruleus has been thought to underly self-stimulation (SS) and the stimulation-bound behaviours (SBB) elicited by electrical stimulation of the dorsal pontine brainstem. However, 6-OHDA-induced lesions of the dorsal norepinephrine bundle that ascends from the locus coeruleus have failed to affect either dorsal pontine SS or SBB in the rat. The present study investigated the hypothesis that the mesencephalic trigeminal nucleus, which lies immediately lateral to the locus coeruleus, mediates dorsal pontine SS and SBB. The mesencephalic trigeminal nucleus is known to have direct excitatory input to the trigeminal motor neurons (MOT V), and accordingly we mapped MOT V for SS and SBB. Supporting the "trigeminal hypothesis" of brainstem SS, electrode placements throughout the trigeminal motor nucleus supported SS. Placements medial to MOT V, in the area of the ascending ventral norepinephrine bundle, failed to support SS, as did placements in the main sensory nucleus. SS in MOT V was shown not to be an artefact of the behaviours elicited by the stimulation.

Moreover, further support for this hypothesis came from results showing that significantly more in vacuo jaw movements were associated with SS placements than with placements which were negative for SS in and around MOT V. In the SBB testing, jaw movements were observed in the inter-stimulation intervals after MOT V stimulation. In contrast, with electrodes placed in the vicinity of the mesencephelic trigeminal nucleus oral behaviours were elicited during the period of the stimulation. The present experiment suggests that motivational functions may occur at the level of cranial motor nuclei.

651 CONTINUOUS LIGHTING ABOLISHES THE NORMAL OSCILLATION OF RETENTION PERFORMANCE IN THE RAT. Daniel P. Weingarten* and Frank A. Holloway. Dept. of Psychiatry and Behav. Sci., Univ. of Okla. Health Sciences Center, Oklahoma City, Ok. 73190.

In this laboratory, Holloway and Wansley (Science, 180: 208, 1973) demonstrated that rats trained on a step-up active avoidance task to a criterion of 4 successive avoidances showed good retention performance at 15 min, 12 hr, and 24 hr, with significantly poorer performance at 6 hr and 18 hr. These authors called this phenomenon the multiple retention deficit (MRD). Animals were individually housed under a 12 hr light-12 hr dark cycle (12-12 group) for 2 weeks prior to training. These results were interpreted in terms of a state dependent hypothesis concerning retrieval processes which are under the control of an unspecified physiological rhythm(s).

Since it has been established that lighting conditions can affect the normal rhythmicity of a number of well known behavioral, hormonal, and biochemical rhythms, we decided to investigate the manner in which continuous bright illumination (CI) might influence the MRD. A control 12-12 group was employed. The control group's data replicated the earlier findings, while the CI group showed no significant differences between the 6 hr, 12 hr, and 24 hr groups, or between the 12 hr, 18 hr, and 24 hr groups. However, the 6 hr group performed significantly worse than the 18 hr group. Independent analysis of 24 hr water intake for both groups showed that the 12-12 group displayed a strong circadian rhythm with 85% water intake during the dark period, while the CI group showed no indications of a circadian function for this measure.

In summary, the results support the hypothesis that the MRD depends upon some unspecified biological rhythm(s). Specifically, housing under constant light conditions alters the circadian pattern of water intake and the periodic character of the MRD.

652 AGE-RELATED DEFICITS OF ACQUISITION AND ACTIVITY IN RATS. Otto L. Wolthuis, Dick L. Knook* and Victor J. Nickolson*. Medical Biol. Lab. TNO and Inst. of Exper. Gerontology TNO, Rijswijk, The Netherlands.

Spontaneous motor activity and conditioned suppression of drinking behavior were assessed in female rats of 3, 12, 18 and 30 months (M) age. By punishment and/or reward the animals were trained to drink only during certain light and sound signal periods. The mechanical simplicity of the task made it unlikely that age differences in response speed, activity or motor skill affected the results. Extraneous interference with training was minimized. Spontaneous motor activity of 12 M rats was as low as that of 30 M rats (18 M not tested) and both were significantly ($p_2 < 0.05$) lower than of 3 M rats. The performances of the 3, 12 and 18 M rats in the learning task however, were similar to each other. Although the initial performance of the 30 M group was comparable to that of the younger groups, it became significantly ($p_2 < 0.05$) poorer after one week of training. It is concluded that these acquisition deficits in aged rats which develop beyond the age of 18 months are independent of the animal's reactivity to punishment and are unrelated to their spontaneous motor activity prior to training.

653 EFFECT OF NORADRENERGIC BLOCKADE IN THE AMYGDALA ON RETENTION FOR PASSIVE AVOIDANCE CONDITIONING. M. G. Zetumer*, B. S. Kapp, R. E. Musty, and P. A. Driscoll*. Univ. of Vermont, Burlington, VT. 05401.

Sprague-Dawley rats were prepared with bilateral cannula positioned at the dorsal surface of the amygdala complex. Intracerebral injections (0.5 µl) of adrenergic blockers or the vehicle alone (a Krebs-Ringer phosphate solution) were administered at varying intervals after training in a one-trial step-through passive avoidance task. Retention was measured 24 hrs. after training. While administration of varying doses of phentolamine and tolazoline, both alpha-blockers, had no effect on retention, the beta-blocker dl-propranolol (8.5, 17.0, 34.0, and 136.0 nmoles) produced a dose related decrease in retention. The significant retention deficit obtained at the 34 nmole dose of propranolol was observed to be both time-dependent and stereospecific since neither delay of dl-propranolol injection nor immediate administration of the dextro-isomer of propranolol produced an effect on retention compared to vehicle injected and unoperated control groups. Comparable deficits obtained with another beta blocker, dl-alprenolol, provide additional support for the specificity of a retention deficit following beta-adrenergic blockade within the amygdala. EEG recordings taken from the site of injection following administration of either propranolol or alprenolol did not exhibit any abnormal afterdischarge activity excluding the possibility that these drugs produce retention deficits via spread of abnormal electrical activity to extraamygdala areas. These data are interpreted as support for the involvement of amygdala noradrenergic systems in time-dependent memory processes.

654 THE LOCUS COERULEUS AND MEMORY: RELATION TO ANISOMYCIN-INDUCED AMNESIA IN MICE. Steven F. Zornetzer and Robert Appleton* Dept. Neuroscience, Coll. Medicine, University of Florida, Gainesville, Fl. 32610.

The participation of the principally noradrenergic brain stem region, the nucleus locus coeruleus (LC), in memory processing was investigated. Previous experiments reported at these meetings and published elsewhere (Zornetzer & Gold, Physiol. Behav., 1976, 16: 331) indicated that electrolytic lesion damage to the LC immediately after learning, but not 6 hrs later, extended the susceptibility of the newly-formed memory to ECS-produced retrograde amnesia (RA). These previous experiments indicated that the lability of the newly-formed memory persisted for at least 168 hrs.

The present experiments investigated the generality of this extended lability period. Post-training LC lesions were made to determine if increased lability of newly-formed memory could be demonstrated in response to anisomycin, a protein synthesis inhibitor and memory disruptive agent.

Swiss ICR male mice were administered LC lesions through previously implanted electrodes immediately following training in an inhibitory avoidance task. In order to determine whether LC damage resulted in an extended temporal gradient of memory susceptibility, various regimens of anisomycin administration were begun either immediately or at a number of time intervals following training and LC lesions. Protein synthesis inhibition was measured in separate groups of mice.

The results of these experiments are discussed in terms of the proposed involvement of active protein synthetic systems and catecholamines in both labile and stabile memory as well as in the maintenance of retrievable memory traces.

Monoaminergic Systems

655 ORGANIZATION OF CATECHOLAMINE-CONTAINING CELL BODIES IN CAT HYPOTHALAMUS.
R.Ackermann, C.Demirjian, M.Glusman, C.L.J.Stokman and R.Katzman, New York State Psychiatric Institute and Albert Einstein College of Medicine, New York, N.Y.

Using the Falck-Hillarp histofluorescence method, we have delineated the catecholamine-containing (CA) neuronal groups in cats. Normal cats; newborn, young adult, and adult; and ventromedial hypothalamus (VMH) lesioned adults were employed. We found that the cat is richer in fluorescent cell bodies, and differs more in its organization from the rat hypothalamus, than previously reported.[1,2] The hypothalamic periventricular CA group (A14) extends from chiasmatic to infundibular levels. In both dorsal and ventral periventricular hypothalamic regions, numerous small CA cell bodies were seen. VMH lesioning revealed a dense band of fluorescent periventricular cells immediately ventral to the III ventricle at the level of the supraoptic decussations. We found a well-represented arcuate (A12) fluorescent group which showed enhanced fluorescence after VMH lesioning, suggesting that A12 cells have major projections to non-infundibular targets. Lesioning also revealed that the ventral A11 cell group, located medial to the mamillothalamic tract, is much more extensive than is apparent in untreated cats; its numerous medium-sized multipolar cells are found not only tucked between the mammillothalamic tract and the III ventricle, but also extend as far ventrally as the ventromedial border of the fornix. This group also extends caudorostrally from the anterior border of the mamillary bodies to infundibular levels where it merges with the smaller, more spindle-shaped cells of CA group A13 which lie along the medial portions of the zona incerta; dorsocaudally, A11 is confined to the diencephalon and, unlike rats' A11 groups,[3] does not extend into the anterior aqueductal region. Increased fluorescence in A11 cells after VMH lesioning, together with intense fluorescent fiber "backup" immediately caudal to the lesions, suggest that an "incerto-hypothalamic" tract has been severed, to which the A11 group is an important contributor.[3] Poitras and Parent (1) reported fluorescent cell bodies in the dorsal chiasmatic nucleus following pretreatment with alpha methyl dopa; similarly, we did not see this fluorescent group in normal animals, but after VMH lesioning numerous fluorescent dorsal chiasmatic cells were observed, suggesting that at least some of their projections are descending. Contrary to previous reports,[1,2] we did not observe fluorescent cell bodies in the lateral hypothalamus. In summary, these findings indicate differences between the cat and the rat in the organization of their hypothalamic CA cell groups. More importantly, they show the cat hypothalamus to have an abundance of CA cells, and they suggest that the cat should be considered a subject of choice in catecholamine-neuroanatomy/hypothalamic-function studies. (Supported by NIH grants: MH 13579-02, and NIH NS 09649.)

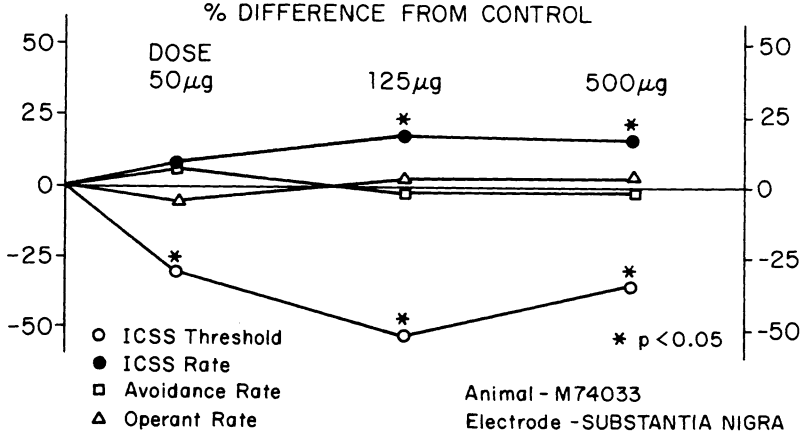
1) Poitras and Parent, J. Morph., 145 (1975) 387-408.
2) Cheung and Sladek, J. Comp. Neurol., 164 (1975) 339-360.
3) Bjorklund, Lindvall, and Nobin, Brain Reseach, 89 (1975) 29-42.

656 AMPHETAMINE FACILITATION OF INTRACRANIAL SELF-STIMULATION BEHAVIOR IN MONKEYS: SPECIFICITY FOR REINFORCEMENT SUBSTRATES. Peter H. Blake* and Douglas M. Bowden. Regional Primate Research Center, and Dept. Pharmacol., Sch. Med., Univ. Wash., Seattle, WA 98195.

The literature suggests that neural substrates of intracranial self-stimulation (ICSS) behavior in rats are catecholaminergic in nature. Pharmacologic studies provide much of the evidence supporting this hypothesis. A common criticism of such studies has been that drug-induced changes in ICSS behavior may be due to nonspecific behavioral arousal or suppression, rather than to specific effects of drugs on neural substrates of positive reinforcement. For example, it has been suggested that facilitatory effects of amphetamine in rat ICSS may be due to general increases in wakefulness or behavioral activity (Roll, Science 169: 1370, 1970).

If amphetamine facilitation of ICSS were due to nonspecific behavioral stimulation, then amphetamine would be expected to facilitate operant responding in general, regardless of the reinforcer used to maintain the behavior. In the present study, a schedule was designed which produced stable, reproducible and comparable rates of lever-pressing in two different and alternating paradigms. In one paradigm, responses were reinforced by positive brain stimulation; in the other, by postponement of a noxious sound stimulus. Both paradigms consisted of one minute time-in periods (during which lever-presses were followed by the appropriate reinforcer), alternating with one minute time-out periods (during which lever-pressing had no consequence). Four dependent variables were compared under control and drug conditions: ICSS rate, ICSS threshold, avoidance response rate and time-out response rate.

Intraventricular d-amphetamine at low to moderate (50-500 µg) doses facilitated ICSS rates and lowered ICSS thresholds. Avoidance and time-out (operant) response rates were unaffected by d-amphetamine over this dose range (Figure 1).



It was concluded that d-amphetamine-induced changes in ICSS behavior were due to drug actions specific for neural substrates of ICSS reinforcement, and not to nonspecific increases in wakefulness or behavioral activity.

This work was supported by USPHS Grant GM00109-15, and USPHS Grant RR00166.

657 LITHIUM PREVENTION OF AMPHETAMINE-INDUCED "MANIC" EXCITEMENT AND OF RESERPINE-INDUCED "DEPRESSION" IN MICE: POSSIBLE ROLE OF 2-PHENYLETHYLAMINE (PEA). R.L. Borison*, H.C. Sabelli, B. Diamond*, P. Maple*, and H.S. Havdala*. (SPON: V. Nair). Mt. Sinai Hosp., Chicago, Ill. 60608, Univ. of Ill., Chicago, Ill. 60680, and Chicago Med. Sch., Chicago, Ill. 60612.

Chronic treatment with LiCl (45 mg/Kg, ip, daily for 8 days) markedly reduces (to 45% of control) the recovery from brain of labelled PEA following ip injection of labelled L-phenylalanine, while decreasing recovery from brain of labelled PEA following ip injection to 63% of control. These effects may account for the therapeutic effect of Li in mania (Sabelli et al., Pharmacologist, 1976). In addition, chronic Li treatment appears to effectively reduce the recurrence of both manic and depressive episodes in affective disorders. Using an activity rating scale (0 to 7), we have now observed in mice that chronic treatment with LiCl (45 mg/Kg, ip, isotonic, daily for 8 days) reduced the jumping, fighting, stereotypias and hyperactivity induced by D-amphetamine sulfate (D-AMPH)(5 mg/Kg, ip); activity rating, 1 hr following D-AMPH: 5.71 after NaCl, 4.91 after LiCl. LiCl also reduced the hypoactivity observed from 1-3 hrs after reserpine (0.75 mg/Kg, 1 hr prior to last LiCl injection); (activity ratings for 1st, 2nd and 3rd hr following reserpine: 2.75, 2.75, and 2 after NaCl; 3.75, 3.5, and 3.4 after LiCl). The table shows the recovery from brain of labelled PEA 10 min after the ip injection of labelled PEA or labelled L-phenylalanine (L-Phen) as % of control:

Treatment	Labelled Precursor	
	L-Phen	PEA
NaCl - Reserpine	304	118
LiCl - Reserpine	78	74
NaCl - D-AMPH	198	87
LiCl - D-AMPH	62	116
LiCl - NaCl	45	63

In NaCl treated mice, D-AMPH appears to increase PEA synthesis and to accelerate disposition [in agreement with previous observations in rabbits suggesting that D-AMPH releases endogenous PEA (Borison et al., Life Sciences, 1974 and 1975)]. Li pretreatment antagonizes the effects of D-AMPH on synthesis and disposition. In NaCl treated mice, reserpine enhanced PEA synthesis and reduced disposition; both of these effects were prevented by Li. These results, together with other evidence for a major role of PEA in the modulation of affective behavior (Sabelli, Chemcal Modulation of Brain Function, Raven Press, 1973; Sabelli and Mosnaim, Amer. J. Psychiat., 1974) indicate that the effects of Li on PEA turnover may contribute to both its therapeutic and prophylactic actions in affective disorders.

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658 SPECIES DIFFERENCES IN MAMMALIAN SIF CELL STRUCTURE AND FUNCTION. Tanemichi Chiba, Christine Heym*, Pushpa Deshmukh*, Asa C. Black, Jr., and Terence H. Williams. Dept. Anatomy, Univ. Iowa, Iowa City, Iowa.

As a result of fluorescence histochemical[1], electronmicroscopic[2], neurophysiological[3], and biochemical[4] investigations, the concept of an inhibitory interneuron in the superior cervical ganglion (SCG) has emerged. This interneuron is a SIF cell, employs dopamine as a neurotransmitter, and hyperpolarizes principal ganglionic neurons by generation of a slow inhibitory postsynaptic potential (s-IPSP). Cyclic AMP is a crucial intermediate in the generation of the s-IPSP and consequent hyperpolarization of the principal ganglionic neuron[4].

Fluorescence and electron microscopic studies of the same sections of SCG from various mammals were carried out by the method of Chiba and Williams[5]. SIF cells were counted and characterized according to the following criteria[6]: Type I SIF cells are solitary, located among the principal ganglionic neurons, and exhibit long fluorescent processes (≤200μ) which ramify among the principal ganglionic neurons; Type II SIF cells are located in clusters close to blood vessels in the subcapsular region or stroma. Processes, when present, are short and head directly towards the nearby blood vessels. In some species, Type II SIF cells are juxtaposed to fenestrated capillaries[7]. We hypothesize that the Type I SIF cell is the true interneuron, while the Type II SIF cell has a neurosecretory function. There are strong indications from fluorescence microscopic appearances that in Tupaia (the tree shrew) virtually all the SIF cells in the SCG are in one or two large clusters and that Type I SIF cells are exceedingly rare.

Species	SIF Cells per mg. Tissue[a]	Percentage SIF Cells Examined[a] Type I	Type II	s-IPSP	DRAC[b]
Cow	5.2 ± 0.1	23.7 ± 5.8%	76.3 ± 5.8%	--	yes
Cat	6.7 ± 1.1	0.481 ± 0.42%	99.5 ± 0.41%	weak[8]	slight
Dog	0.430 ± 0.27	0.690 ± 0.56%	99.3 ± 0.57%	--	--
Tupaia	290	5%	95%	--	--
Monkey	2.3 ± 0.7	48.5 ± 10%	51.5 ± 10%	--	yes
Rabbit	6.9 ± 0.5	74.5 ± 20%	25.5 ± 20%	yes[3]	yes
Rat	280. ± 30	NA[c]	NA[c]	--	?
G. Pig	76.0 ± 20	NA[c]	NA[c]	no[3]	no

[a]Mean ± Standard Error of the Mean. [b]Dopamine Receptor--Adenylate Cyclase
[c]Not Available. Types I and II cannot be distinguished in these ganglia.

The greatest number of SIF cells per mg. tissue were observed in the rat and guinea pig SCG. In the dog SCG SIF cells were either sparse or absent (in 9 of 12 ganglia no SIF cells were observed). The Type I SIF cells were particularly rare in both carnivores. The cat SCG is also noteworthy because the s-IPSP observed in this ganglion is weak[8] and because minimal elevation of cyclic AMP levels occurs when the cat SCG is stimulated with dopamine[9]. Conversely, in the cow and monkey SCG where cyclic AMP synthesis is strongly stimulated by dopamine, an abundance of Type I SIF cells occurs. These results indicate wide species diversity in SIF cell structure and function.

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659 MONOAMINE DISTRIBUTION IN PRIMATE HYPOTHALAMUS. David L. Felten*, Gloria E. Hoffman, and John R. Sladek, Jr. (SPON: W.J. Nikkowitz). Depts. of Anat., Indiana Univ. Sch. Med., Indianapolis, IND. 46202 and Univ. Rochester Sch. Med. and Dent., Rochester, N.Y. 14642.

Catecholamine (CA) varicosity distribution in the hypothalamus of 20 adult and juvenile Macaca mulatta (rhesus monkey) and 18 adult and juvenile Saimiri sciureus (squirrel monkey) was examined by the histochemical fluorescent technique for density, intensity, and size of the varicosities. Marked similarities and differences were noted in CA distribution within specific hypothalamic nuclei of rhesus and squirrel monkeys.

Anterior Region. The preoptic periventricular area contained high densities of CA varicosities in both species, with the highest density in the ventral portion. Medial and lateral preoptic areas contained moderate densities of CA varicosities in both species. The suprachiasmatic nucleus in rhesus monkey possessed scattered CA varicosities, but in squirrel monkey showed no CA terminals. Abundant serotonin varicosities were seen in this nucleus in both primates. The nucleus supraoptic diffusis (retrochiasmatic area) showed a high density of fine CA varicosities in squirrel monkey, and a sparse density in rhesus monkey. The anterior hypothalamic nucleus contained only scattered CA varicosities in both primate species.

Middle Region. The paraventricular, periventricular, and dorsomedial nuclei all contained high densities of CA varicosities in both primates, while the dorsal hypothalamic and perifornical areas contained moderate densities. The ventromedial nucleus contained sparse CA varicosities. The lateral hypothalamic area contained sparse varicosities, with small areas of marked density noted in squirrel monkey. The arcuate nucleus contained a high density of CA varicosities in rhesus monkey, and a lesser density in squirrel monkey. The median eminence contact zone showed marked rostral-caudal and medial-lateral intensity changes in both primates, most strikingly noted in rhesus. The caudal contact zone was most intense, with diffuse fluorescence. A gradient of caudal intensity was seen in the perivascular region of the median eminence.

Posterior Region. Posterior periventricular, posterior hypothalamic, and premammillary nuclei demonstrated moderate densities of CA varicosities, while tuberolateral and tuberomammillary nuclei showed virtually no fluorescence in both primates. The rhesus mammillary complex was practically devoid of CA varicosities, but the lateral mammillary nucleus in squirrel monkey showed a moderate density.

Similar densities of abundant CA varicosities were seen in both primates in supraoptic, paraventricular, preoptic periventricular, and dorsomedial nuclei. But clear dissimilarities in varicosity distribution were seen in medial preoptic, suprachiasmatic, retrochiasmatic, arcuate, and lateral mammillary nuclei and in median eminence. Many primate hypothalamic nuclei contained dissimilar varicosity patterns and densities from those reported in cat and rat. The regional CA innervation of the median eminence and intense CA innervation of the arcuate, periventricular, supraoptic, and paraventricular nuclei suggest a prominent role for CAs in neuroendocrine regulation of the anterior and posterior pituitary in primates. CA distribution in additional hypothalamic regions suggests a CA role in regulation of visceral functions in primates. (Supported by Program Project Grant NS-11642 and a Showalter Foundation Grant).

660 CATECHOLAMINE DENERVATION OF LIMBIC FOREBRAIN AND ANTEROMEDIAL STRIATUM PRODUCES DEFICITS IN LOCOMOTOR EXPLORATION. J. Stephen Fink*, Brian J. Greenfield* and Gerard P. Smith. Dept. of Psychiatry, Cornell Univ. Med. Coll. and E.W. Bourne Behavioral Research Laboratory, The New York Hospital-Cornell Medical Center, White Plains, N.Y. 10605.

Bilateral injections of 6-hydroxydopamine (6-OHDA) in the antero-lateral (AL) hypothalamus produced severe catecholamine (CA) denervation of wide areas of the rat forebrain, including neocortex, hippocampus, limbic forebrain, anteromedial striatum and lateral hypothalamus. Bilateral 6-OHDA injections in the anteromedial (AM) hypothalamus produced severe CA denervation only in neocortex, hippocampus and AM hypothalamus. AL or AM 6-OHDA injections left the CA innervation to amygdala, thalamus, posterior striatum and brainstem generally intact (Neurosci. Abst. 1:406, 1975). When tested for locomotor exploration (LE) in an open field (OF) beginning 15 days after surgery AL, but not AM, 6-OHDA rats moved and reared less than their respective vehicle-injected controls. This suggested that loss of CA within limbic forebrain, anteromedial striatum and lateral hypothalamus that was present in AL, but not in AM, rats was the area of denervation necessary for the deficits in LE observed in AL 6-OHDA rats.

These denervated regions are rich in dopaminergic (DA) terminals. To test the hypothesis that DA denervation in these areas is critical for the deficit in LE, rats were pretreated with desmethylimipramine (DMI, 25 mg/kg i.p.) 30 min. before the AL 6-OHDA injection. Such pretreatment protects noradrenergic (NA) fibers, but not DA fibers, from the toxic effects of 6-OHDA injected into other brain areas (Price and Fibiger, Br. Res. 99:189, 1975). The DMI-AL 6-OHDA rats showed the same deficits in the OF as AL 6-OHDA rats; DMI-AL 6-OHDA rats moved less and reared less than vehicle-injected rats (see Table). Histofluorescent analysis of 3 DMI-AL 6-OHDA brains by the glyoxylic acid-Vibratome method revealed the presence of parietal cortical, hippocampal and lateral hypothalamic CA fibers, and severe CA denervation in limbic forebrain (nuc. accumbens, olfactory tubercle, dorsal bed nuc. stria term., and frontal, anterior cingulate and piriform cortices) and anteromedial striatum. The presence of CA fibers in parietal cortex and hippocampus indicates that DMI pretreatment protected NA fibers from 6-OHDA under our conditions. Thus, the loss of CA fibers in limbic forebrain and anteromedial striatum may be interpreted as loss of DA fibers. These results are consistent with the hypothesis that DA denervation within limbic forebrain and/or anteromedial striatum is the critical lesion for the deficits in locomotor exploration observed in the OF. The contribution, if any, of NA denervation to the LE deficits remains to be demonstrated.

	Locomotion	Rears
AL Vehicle n=9	220 ± 35	26 ± 2
AL Vehicle DMI n=6	259 ± 23	29 ± 6
AL 6-OHDA n=12	140 ± 19**	9 ± 2**
AL 6-OHDA DMI n=8	115 ± 23*	8 ± 3^{+}

($\bar{X}$ ± SEM, squares crossed and rears per 5 min. on the initial trial in the OF, * $p<0.01$, ** $p<0.001$, $^{+}$ $p<0.0025$, Student's t-test, two-tailed, compared with vehicle-injected control)

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661 EFFECTS OF PCPA, TRYPTOPHAN, AND A TRYPTOPHAN-FREE DIET ON MOUSE KILLING BEHAVIOR OF RATS. Judith L. Gibbons*, Gordon A. Barr, Wagner H. Bridger, and Sarah Fryer Leibowitz. Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, New York, 10461, and The Rockefeller University, New York, New York 10021.

The role of serotonin (5-hydroxytryptamine, 5-HT) in mouse killing by rats was investigated by using para-chlorophenylalanine (PCPA) injections and maintenance on a tryptophan-free diet to deplete brain serotonin and by l-tryptophan injections to increase brain serotonin.

As reported by others, PCPA, at relatively high doses (300 - 400 mg/kg, induced killing in one-third of nonkiller rats. At lower doses (75-150 mg/kg, killing was significantly facilitated in killers in two different test situations. In the home cage, PCPA blocked the satiation of killing normally exhibited by rats with repeated prey exposure. In a novel environment, PCPA decreased the rats' latency to attack a mouse. The time course of PCPA's blocking of satiation appeared to parallel the time course of PCPA induced depletion of brain serotonin. Specifically, at 2 days after administration of 150 mg/kg PCPA, killing was facilitated and brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels were significantly reduced by 60% and 58% respectively. At 8 and 14 days after injection, neither mouse killing behavior nor brain levels of 5-HT and 5-HIAA differed significantly from preinjection levels. The facilitation of mouse killing by PCPA was reversed by injections of 5-hydroxytryptophan (100 mg/kg), the immediate precursor of serotonin. At a dose that facilitated mouse killing, PCPA did not alter the topography of the killing response, nor did it induce rat pup killing or general irritability. Food and water intake and open field activity were somewhat decreased by PCPA (150 mg/kg).

In contrast to PCPA's facilitation of mouse killing, injections of l-tryptophan (100 mg/kg), the amino acid precursor of serotonin, lengthened the rats' latencies to attack and kill the mice, and produced a concomitant increase in brain serotonin (37% and 5-HIAA (73%). Lower doses of l-tryptophan (25 - 50 mg/kg) were ineffective in suppressing killing. At the 100 mg/kg dose, l-tryptophan produced a small decrease in open field locomotion.

Short-term maintenance on a tryptophan-free diet, which decreased brain 5-HT by 40% and 5-HIAA by 40%, dramatically facilitated killing behavior in killer rats. Moreover, the diet also tended to induce killing in non-killer rats. The facilitated or induced killing response observed in rats on the tryptophan-free diet had a similar topography to that of the natural killing response. When l-tryptophan (0.5% or 2.0%) was added to the tryptophan-free diet, both behavioral and biochemical effects of the diet were reversed. The 0.5% l-tryptophan diet restored brain 5-HT to normal levels and increased 5-HIAA by 43% while the 2.0% l-tryptophan diet increased brain 5-HT and 5-HIAA by 30% and 38% respectively. Under both conditions, killing behavior was at normal control levels.

These results are generally consistent with the working hypothesis that brain serotonergic systems have an inhibitory effect on mouse killing by rats.

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662 β-ADRENERGIC RECEPTOR AND CATECHOLAMINE-STIMULATED ADENYL CYCLASE IN NORMAL AND DENERVATED SKELETAL MUSCLE SARCOLEMMA. Stuart P. Grefrath*, Peter B. L. Smith* and Stanley H. Appel. Div. Neurol. and Dept. Biochem., Duke Med. Ctr., Durham, NC 27710.

Denervation results in well-defined physiological changes in surface membrane acetylcholine receptor in mammalian muscle. To define the possible biochemical changes in other receptor functions, the β-adrenergic receptor and adenylate cyclase were studied in surface membranes from 1, 2, 3, 5, and 7 day denervated rat skeletal muscle. Our initial observations demonstrated that sodium fluoride stimulated adenyl cyclase decreased 50% by day 5, and all subsequent data were derived from 5 day denervated membranes. Isoproterenol stimulated adenyl cyclase showed half activation (A [1/2]) for normal and denervated membranes at concentrations of 1.2×10^{-7}M and 2.0×10^{-7}M, respectively. Identical experiments employing the guanine nucleotide GppNHp resulted in a (1) shift of A[1/2] to 1 to 3×10^{-8}M and (2) two-fold increase in isoproterenol stimulated activity. In each case, hormone stimulated adenyl cyclase was decreased by 55-65% in denervated membranes. The reaction conformed to a β stimulated response since studies on the potencies of other catecholamines showed the following relationship for both normal and denervated membranes: Isoproterenol>Epinephrine>Norepinephrine.

To determine whether the observed change in adenyl cyclase activity after denervation is coincident with a change in the β-adrenergic receptor, we utilized the binding of [^{3}H] (-) Dihydroalprenolol to characterize the β-adrenergic receptor in sarcolemma. The interaction of [^{3}H] (-) Dihydroalprenolol with purified skeletal muscle sarcolemma is completely described by the thermodynamic expression $\bar{\nu} = nKL/(1+KL)$ with n (1.39×10^{-9}M/gm) number of noninteracting sites and association constant k (3.8×10^{9}L/M). Competitive studies with other adrenergic ligands indicate the stereospecificity and order of affinity characteristic of the β-receptor, i.e., (-) alprenolol $\tilde{>}$ (-) propranolol > (+) propranolol > epinephrine $\tilde{>}$ isoproterenol > norepinephrine > phentolamine. Identical results were obtained with Dihydroalprenolol binding to 5 day denervated membranes. Thus, despite the decrease in isoproterenol activation of adenyl cyclase, the thermodynamic characteristics of the receptor do not change after five days of denervation. Given the known function of hormones in the regulation of cellular metabolism, these results offer potentially useful guides for exploring the initial events of altered muscle metabolism in the denervated state. Supported in part by grant NS07872 and grant NS12213 from NINCDS.

663 CONCENTRATION OF SEROTONIN AND TRYPTOPHAN IN SYNAPTOSOMES DERIVED FROM A PERIPHERAL SEROTONERGIC NEURON. G. M. Jonakait* and M.D. Gershon. Dept. Anat., Columbia University, College of P&S, New York, N.Y. 10032.

Intrinsic serotonergic neurons have been identified in the myenteric plexus of the mammalian gut. These neurons contain tryptophan hydroxylase and can convert ^{3}H-tryptophan (Try) to ^{3}H-serotonin (5-HT). They also take up 5-HT by a specific high affinity mechanism. This study was done to determine if the uptake of 5-HT is a property of the terminals of these neurons and if the terminals also take up the 5-HT precursor, Try. Strips of longitudinal muscle with adherent myenteric plexus (LM-MP) were dissected from the guinea pig ileum and incubated for 30 min with ^{3}H-5-HT (0.9 μM). After a 30 min wash the tissue was homogenized in 0.3M sucrose. The homogenate was subjected to differential and discontinuous density gradient centrifugation. The following fractions (P= pellet; S=supernatant) were obtained: P1 (1000 x g x 10 min), P2 + S2 (100,000 x g x 60 min) and gradient fractions derived from P2: P2A, P2B, and P2C, found at the interfaces between .3M and .6M, .6M and 1.2M, and 1.2M and 1.6M sucrose respectively. P2B contained synaptosomes when examined by electron microscopy, and synaptosomes of brain, homogenized as carrier together with gut, were also located in this fraction. P2A contained a variety of membranous vesicles and P2C contained mitochondria, fragments of muscles, and some synaptosomes. Much of the radioactive 5-HT was soluble; ^{3}H-5-HT in S2 was 64% of the total in S2 + P2. Of the particulate radioactivity in P2, the largest amount was found in P2B (44.9 ± 2.7%SE) while lesser amounts were contained in P2A (26.1 ± 2.5%) and P2C (28.8 ± 2.7%). Addition of brain as carrier increased P2B to 65.8% at the expense of P2C. Reserpine (2.5 mg/kg) pretreatment of animals decreased the particulate radioactivity in P2 and lowered the specific activity of P2A and P2B. Hypotonic shock released much of the radioactivity from P2B to the supernatant but some ($\sim$13%) was found in a vesicular fraction. Intact LM-MP took up ^{3}H-Try; the Km was 47 μM. Upon homogenization and centrifugation with carrier brain as described above, over 90% of the ^{3}H-Try was in S2. Of the particulate radioactive Try derived from P2, most was in P2B (88%), while the rest was in P2A (12%); P2C contained less than 0.1%. These experiments indicate that both ^{3}H-5-HT and ^{3}H-Try are taken up by axonal terminals and thus are concentrated in synaptosomes derived from these axons.

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664 THE SEROTONIN INNERVATION OF RAT LOCUS COERULEUS: AXON TERMINALS WITH A SPECIAL TYPE OF PRESYNAPTIC ORGANELLE. Lucienne Léger* and Laurent Descarries. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec.

Fluorescence histochemical, and immuno histo- and cytochemical studies have established that the locus coeruleus (LC) of rat is entirely built of norepinephrine-containing neurons and is innervated by serotonin (5-HT) fibers. In order to identify these afferents and be able to study their distribution and fine structural features, high resolution radioautography was used following administration of 5-HT-^{3}H in animals pretreated with a monoamine oxidase inhibitor.

After prolonged ventricular perfusion with 10^{-5} or 10^{-4} M 5-HT-^{3}H, or local instillation of the tracer (ca. 100 µCi in 0.5 µl) in the vicinity of the nucleus, light microscope radioautographs showed numerous silver grain aggregates, typical of intensely labeled axonal varicosities, in the periventricular gray substance and the LC. In both regions, comparable numbers of reactive sites were detected after 10^{-5} and 10^{-4} M ventricular perfusions with or without concomitant administration of non-radioactive norepinephrine at a concentration 10 times higher than that of 5-HT-^{3}H, and following either 15 or 30 days of radioautographic exposure. After intraventricular but not intratissular 5-HT-^{3}H, strong labeling of supra-ependymal 5-HT fibers was also observed.

In electron microscope radioautographs of the LC, all reactive varicosities, and only these, exhibited a special type of organelle, in the form of microvesicles and/or canaliculi 15-25 nm in diameter, which were found in lieu of synaptic vesicles and usually associated with several large dense-core vesicles. Grouping of these organelles conferred a greater density to labeled than unlabeled varicosities. This was further enhanced by the presence of an electron opaque material which partially filled many of the microvesicles and canaliculi. In fact, even though the specimens had been fixed by using a conventional phosphate buffered aldehyde and osmium sequence, these organelles were strongly reminiscent of the so-called tubular reticulum, described by Tranzer and Richards ('72 and '75) as an amine storage site within the perikaryon and dendritic and axonal processes of noradrenergic ganglion cells fixed with a sequence of aldehydes, dichromate and osmium, or in chromate-dichromate buffered aldehydes.

In the LC, the labeled varicosities could be seen to make asymmetrical synaptic contacts on dendritic processes but not on the nerve cell bodies. Similar axonal profiles were visible in the periventricular gray and supra-ependymal plexus, but the latter failed to show synaptic junctions.

This investigation therefore reveals the existence of central 5-HT nerve endings endowed with highly characteristic structural features presumably related to their monoamine storage capacity. Such axonal varicosities have also been found in the cerebellum (Chan-Palay and Descarries '75), and all those of this type might belong to collateral arborizations arising from the same parent nerve cell bodies. In the LC, these peculiar terminals subserve direct synaptic, as well as possible non-synaptic relationships between 5-HT afferents and norepinephrine neurons.

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665 DISTRIBUTION AND DEVELOPMENT OF CATECHOLAMINES IN PRIMATE NEOCORTEX.
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Recent evidence from investigations in rats have suggested that dopamine (DA) is a neurotransmitter in those cortical regions which receive projections from the dorsomedial nucleus of the thalamus and which can be regarded as the rodent homologue of the prefrontal association cortex in the monkey. However, little information exists on catecholamine levels in specific regions of primate neocortex. Accordingly, the distribution of norepinephrine (NE) and DA was analyzed fluorimetrically in 4 regions of cerebral cortex of 2-day old, 8-mo. old, and 2-3 year old rhesus monkeys. At all ages investigated, microgram per gram concentrations of both NE and DA in cortex show a rostral-caudal distribution. The highest concentrations are found in orbital and dorsolateral prefrontal cortex while the lowest are found in striate cortex. In young adults, the DA concentration in both prefrontal areas is 65% of NE compared to ratios of 35% and 15% for parietal and striate cortex, respectively. For all areas examined, NE shows a graded increase with age. In contrast, DA concentration in dorsolateral prefrontal cortex is high in the newborn, even exceeding that of NE. Similarly, in the orbital prefrontal cortex, DA exists in high concentrations at birth. DA in both prefrontal regions declines at 8 mo. of age and then rises again in the young adult. This pattern of DA development in prefrontal cortex differs from that of other cortical regions. In parietal cortex, DA level increases from birth to 8 mo. of age but no further change occurs between 8 mo. and 2-3 years, while striate cortical DA remains at the same low level from birth to maturity. To other lines of evidence for DA as a neurotransmitter in prefrontal cortex may be added the present findings: (1) in young adult monkeys DA exists in higher concentrations in prefrontal cortical areas than in posterior association and primary sensory cortex. (2) DA and NE in prefrontal cortex exhibit different profiles of development indicating that their concentrations may arise independently from two separate systems.

666 AFFERENT CONNECTIONS OF THE NUCLEUS RAPHE DORSALIS AND THE OCULOMOTOR COMPLEX IN THE RAT. Daniel Pasquier*, William B. Forbes and Peter J. Morgane (SPON: H. Hoagland). Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

The nucleus raphe dorsalis (RD) is a very elaborate and conspicuous structure in the lower mesencephalon, which has been in the last decade a key-stone in many schemes for functional phenomena. At present there are contradictory theories as to its function. This may be explained, in part, because 1) the RD is placed in the ventral portion of the central gray and its topographical relation with neighboring structures, such as the oculomotor complex (OMC), trochlear, and dorsal tegmental nuclei of Gudden (DT) make difficult or impossible a successful approach using classic anatomical and physiological techniques; 2) very little is known about its internal structure, which is far from being a simple and uniform neuronal organization; and 3) especially in the rat, very little is known of its connections. Nauta (1958) reported, in the cat, descending projections from prosencephalic structures to RD region. In the rat, Morest (1961) found terminal degeneration in RD after lesions of the dorsal tegmental nucleus and dorsal longitudinal fasciculus.

In the present studies, the RD and OMC afferents were examined by injections of horseradish peroxidase (HRP) in RD or OMC. Since the limits of RD are not precisely defined, "afferents to RD" implies afferents to both RD and the ventral portion of the central gray surrounding DR.

After HRP injections in RD, labeled neurons were found in the following nuclei; lateral habenular, interpeduncular, substantia nigra, ventral central gray, cuneiformis (its rostral portion), dorsal parabrachial, dorsal tegmental of Gudden, reticularis pontis oralis and caudalis, gigantocellularis (ventromedial portion), reticularis paramedianus, lateral reticular, raphe magnus and raphe obscurus.

After HRP injections in OMC, labeled neurons were found in the following nuclei: pretectal reticular formation, Cajal's interstitial, Darkschewitsch's accessory, substantia nigra, Tsai's ventrotegmental area, nucleus cuneiformis, red, deep layers of superior colliculus, reticularis tegmenti pontis, supragenualis (rostral end of the nucleus prepositus hypoglossi) and medial vestibular. These data demonstrate widespread, but specific, projections from the so-called accessory oculomotor nuclei, with some notable differences from those reported in the cat, i.e., there were no labeled neurons in the nucleus abducens. Also, it is worth noting that labeled neurons were found in the nucleus reticularis tegmenti pontis, which may be implicated in the control of eye movements.

The distribution of labeled neurons after injections in the RD suggests a complex pattern of afferents from non-specific nuclei at all levels of the brainstem. These results indicate that the nucleus raphe dorsalis receives multiple reticular inputs from a variety of brainstem areas, which, in large part, correspond to aminergic and cholinergic fields as demonstrated by histofluorescence mapping and histochemical analysis.

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667 THE RELEASE OF SEROTONIN, DOPAMINE AND NOREPINEPHRINE FROM THE CEREBRAL CORTEX IN THE CAT. Tomas A. Reader*, Lise Farley*, Jacques de Champlain and Herbert H. Jasper. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec, Canada.

In previous studies from our laboratory we have measured the liberation of norepinephrine (NE) and dopamine (DA) in the cerebral cortex using a superfused cortex preparation and a sensitive and specific radioisotopic method. In addition we have proposed an interaction between catecholaminergic (CA) systems and cholinergic systems in which acetylcholine (ACh) released by specific sensory mechanisms and/or general arousal could modulate CA release through a presynaptic mechanism. The purpose of the present group of experiments was to determine whether endogenous serotonin (5-HT) is released from the cerebral cortex in the cat. In addition an attempt was made to correlate the rates of release of 5-HT, NE and DA with the state of cortical activation. Finally CA were also measured in peripheral blood during the periods of cortical superfusate collections.

Special nylon chambers (superfusion "cups" or cylinders) were placed on the cortical surface as previously described (G.C. Celesia & H.H. Jasper, J. Neurol., 16: 1053, 1966; J.F. Mitchell, J. Physiol., 165: 98, 1963; T.A. Reader et al., Brain Res., 1976, in press). Each sample was collected over a 30 min period in tubes placed on crushed ice and as soon as they were collected the superfusates were separated in two aliquots, acidified and maintained frozen until the assays were performed. The CA content was determined by the radiometric enzymatic method of J.T. Coyle & D. Henry (J. Neurochem., 21: 61, 1973) following the modification of de Champlain et al., (Circul. Res., 38: 109, 1976). For 5-HT the superfusates were assayed with the radiometric enzymatic method of Saavedra et al., (J. Pharmacol. exper. Ther., 186: 508, 1973).

In the flaxedilized animal maintained under local anaesthesia the concentration of CA was fairly constant in a given experiment under basal conditions, but varied greatly from one experiment to another. For the visual cortex the average basal release of NE in 18 experiments was 20.09 ± 3.64 pg/min/cm^2 while the average for DA was 34.01 ± 7.62 pg/min/cm^2. For the somatosensory cortex the average release of NE was 25.80 ± 8.91 pg/min/cm^2 (n = 6) while the average for DA was 55.52 ± 12.28 pg/min/cm^2 (n = 6). We were unable to establish any consistent correlation between the EEG pattern and the differences in release of NE or DA in the resting state in the animals studied up to date. However during slow wave sleep (SWS) there was a rapid decrease in NE release followed later by a decrease in DA. The variations in 5-HT release were greater than for the CA (the range was from 0.0 to 5 ng/min/cm^2). However the greatest values for 5-HT release were measured during SWS, when NE release is low and DA remains at a steady level or is decreased. The cortical endogenous content for NE was 0.261 ± 0.013 µg/g (n = 20), for DA 0.174 ± 0.012 µg/g (n = 20) and for 5-HT 0.150 ± 0.034 µg/g (n = 20).

These results show: 1) that measurable amounts of 5-HT can be assayed in cortical superfusates; 2) that there is no correlation between the circulating NE and DA and the CA released into the superfusates; and 3) that 5-HT, NE and DA are independent neurotransmitters but that the degree of cortical excitability may be due to a critical equilibrium between NE, DA, 5-HT and other neurotransmitters such as ACh and GABA.

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668 BEHAVIORAL CHANGES FOLLOWING LESIONS OF THE LOCUS COERULEUS IN MACACA ARCTOIDES. D. E. Redmond, Jr., Y.H. Huang*, D.R. Snyder, J.W. Maas, J. Baulu*, Dept. of Psychiatry, Yale University Sch. Med., New Haven, Ct. 06510.

Small lesions of the pontine nucleus locus coeruleus (LC) have been shown to produce decrements in brain norepinepherine (NE) in certain areas, without changes in other biogenic amines or neurotransmitters in rodents. We now report the effects of such lesions in behavior, including natural social behaviors of an old world primate species. 10 adult female Macaca arctoides were studied in 2 sequential experiments. In each study, the same adult male was caged with 5 females for 1 month and allowed to stabilize as a social group. Each female was then observed for 100 observation points at the same time each day during 13 days of the next month, using a focal animal-time sampling technique. Animals were then individually caged, and the LC was bilaterally lesioned in 3 females in each group, while the other 2 were sham operated. 18-25 days after the LC lesioning procedure the animals were replaced in their social groups for an additional 1300 observations per monkey. At the conclusion of the experiment the histological sections showed the lesions to be in the LC in all 6 lesioned animals. Biochemical studies confirmed large decrements in NE in 4 of the animals with no change in dopamine concentration. The remaining 2 animals died unexpectedly during the study, and NE concentrations were not obtainable.

Data from both experiments were combined for statistical analysis of the remaining 4 lesioned monkeys vs. the 4 controls. Significant increases in quantitative behaviors in LC lesioned monkeys included: eating, drinking, spatial displacement of other monkeys, threats, physical attacks, horizontal and body movement and time spent greater than an arm's reach from any animal (alone). Several behaviors decreased including: huddling, giving or receiving grooming, self grooming, self mouthing, scratching and time in physical contact with others. Some changes occurred also in the sham-treated monkeys which were secondary to those in lesioned animals: e.g. receiving displacements, receiving threats, etc.

These changes were thought to result from loss of function of a specific NE system in brain. They are in apparent contradiction to what might be expected to result from drastic decrements in NE on the basis of the catecholamine hypothesis (Schildkraut and Kety, 1967) or previous studies with AMPT or 6-hydroxydopamine in primates (Redmond et al, 1971; Redmond et al, 1973). They are consistent however with our current findings from electrical stimulation of this area (Redmond et al, in press) and some previous paradoxical pharmacological data. These findings should contribute to further hypotheses concerning the role of the LC system in normal and pathological behaviors.

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669 STRAIN DEPENDENT DIFFERENCES IN TYROSINE HYDROXYLASE IN INBRED MICE ARE DUE TO DIFFERENCES IN NUMBER OF DOPAMINERGIC NEURONS. R.A. Ross, A.B. Judd, 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 if the difference in the activity of the enzyme tyrosine hydroxylase (TH) in the whole brain between two strains of inbred mice, CBA/J and BALB/cJ (Ciaranello et al., Life Sci.11:565-572, 1972), was attributable to selective differences in TH activity in dopaminergic (DA) and noradrenergic neurons and, if so, through what mechanism. TH was assayed regionally in brain areas containing DA cell bodies (substantia nigra, SN) and terminals (caudate nuclei, olfactory tubercle) and noradrenergic cell bodies (locus coeruleus, LC). Dopamine-β-hydroxylase (DBH) was assayed in LC and in noradrenergic terminals of the hypothalamus. TH activity was lower by 45% ($p<.001$) in the SN and 25-30% lower in DA terminals of CBA/J mice. In contrast, there were no strain differences in TH or DBH activities in LC or in DBH activity in hypothalamus. Immunochemical titration with a specific antibody to TH demonstrated that the strain difference in TH activity in SN was entirely due to a difference in the amount of enzyme protein. Counts of neurons in SN, immunochemically stained for TH by the peroxidase-antiperoxidase method, demonstrated that the number of TH-containing cell bodies was 49% less in CBA/J mice (1664 vs. 3384, $p<.001$). In contrast, there was no difference in the number of TH-containing cells within the LC of these strains (629 in CBA/J and 624 in BALB/cJ). The calculated enzyme activity/stained SN cell was 0.67 picomoles/h/cell in CBA/J and 0.75 picomoles/h/cell in BALB/cJ mice, an insignificant difference.

We conclude that the strain-dependent differences in brain TH activity between CBA/J and BALB/cJ mice are due to differences in the number of enzyme molecules and is entirely attributable to a difference in the number, but not the type, of DA neurons.

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670 DIFFERENTIAL CATECHOLAMINERGIC AND BEHAVIOURAL EFFECTS OF 6-HYDROXYDOPAMINE ADMINISTERED AT DIFFERENT AGES TO NEONATAL RATS. Matti Saari* and Bruce A. Pappas. Dept. of Psychol., Carleton U., Ottawa, Ontario, K1S 5B6

Neonatal rats were injected with 6-hydroxydopamine (6-OHDA) or vehicle on days 1, 5 or 10 of life. Injections were 2 x 50 mg/kg, 12 hrs apart administered sc. As shown in the table, Ne levels assessed at 70 days of age indicated that maximal depletion of peripheral (heart) Ne was observed after injections at 10 days while maximal brain Ne effects were observed after injections at 1 day of life. After the latter, Ne levels of terminal fields arising from the locus coeruleus and innervating cortical and forebrain limbic structures were profoundly reduced as was the level in the spinal cord. Conversely Ne levels were highly elevated in the pons and less so in the cerebellum,hypothalamus, midbrain and medulla. Dopamine (Da) level in the striatum was unaffected by the treatments but day 1 injections elevated Da levels in many brain parts, particularly the amygdala where Da was 163% of control value.

Tissue	Ne level (% Veh) 1	5	10
Spinal Cord	41	137	123
Cerebellum	132	72	77
Cortex: Temporal	38	95	93
Remainder	17	63	99
Olfact. Lobe	84	112	108
Hippocampus	31	46	73
Septum	85	135	139
Amygdala	44	99	112
Striatum*	104	104	112
Thalamus	98	99	101
Hypothalamus	128	122	99
Mid brain	142	97	94
Pons: Dorsal	183	124	97
Ventral	210	130	120
Medulla	135	102	96
Heart	56	29	20

* Da level

Multiple behavioral deficits were observed only in the rats injected on day 1. Tested at repeated intervals from 15 to 60 days of age, these rats performed poorly on a battery of tests of motor ability, although their gross activity levels at these times were normal. Clonic convulsions at decapitation were also absent. Startle response to loud tone was normal as was pain sensitivity and morphine analgesia assessed by the hot plate technique. However, these rats failed to normally suppress previously trained water drinking when the latter was punished by painful foot shock. These data indicate early and persisting deficits after 6-OHDA on day 1 of life, suggesting a lack of functional brain plasticity after early damage to the dorsal Ne forebrain terminal field and analagous to that observed by Altman's laboratory after early x-ray irradiation of the CNS. Furthermore this damage has some effects similar to those reported after dorsal bundle lesion in the adult in that both cause deficits in procedures requiring adaptation of learned behaviors to altered environmental contingencies. We suggest that the forebrain dorsal Ne field is necessary not for learning per se but for the effective clearance of learned response programmes determining ongoing behavior when established reward/punishment contingencies are suddenly altered.

671 SEROTONIN CONTAINING PERIKARYA AND PATHWAYS IN THE STUMP-TAILED MACAQUE (MACACA ARCTOIDES). John R. Sladek, Jr. and David L. Garver. Dept. Anat., Univ. Rochester Sch. Med., Rochester, NY. 14642, Illinois State Psychiatric Institute, Chicago, IL. 60612.

Serotonin (5-HT) histofluorescence is often difficult to obtain with formaldehyde-induced histochemistry and for this reason the 5-HT systems in the brain have received less attention neuroanatomically than the catecholamine systems. Previously (Sladek, Tabakoff and Garver, Brain Res. 67:363-371, 1974) we reported 5-HT histofluorescence of high intensity in the brain stem of neonatal macaques, wherein its demonstration did not require pharmacological pretreatment or methodological variation of the Falck-Hillarp technique.

We have used this model to examine the distribution of 5-HT perikarya and pathways in the brain of the stump-tailed macaque. Neonates (1-7 days old) and infants up to 12 weeks of age were prepared for formaldehyde-induced fluorescence histochemistry. Brain sections were examined in a modified Leitz MPV-2 microspectrofluorometer and suspected 5-HT loci were mapped and spectrally analyzed. Qualitative microspectrofluorometric analysis was performed according to methods outlined and developed in the laboratory of Anders Björklund. Cells suspected of containing 5-HT, as viewed with narrow band excitation and low cut off emission filters, were spectrally analyzed and found to exhibit 5-HT emission peaks at 520nm. Such cells contained an intense yellow histofluorescence which extended into neuronal processes. In many instances a single 5-HT cell contained more than one fluorescent process, perhaps indicating a dendritic compartmentalization of 5-HT as reported earlier for catecholaminergic perikarya (Sladek and Parnavelas, Brain Res., 100: 657-662, 1975). Serotonergic perikarya were seen within the brain stem raphe at medullary, pontine and mesencephalic levels; although no 5-HT perikarya were seen rostral to inferior collicular levels. Thus, 5-HT neurons as reported in the red nucleus of the cat were not visualized in the stump-tailed macaque. At medullary levels the nucleus raphe pallidus and nucleus raphe obscuris contained large numbers of 5-HT perikarya and corresponded roughly to the B1 and B2 groups described in the rat. At pontine levels the nucleus raphe magnus and the caudal pole of the nucleus raphe dorsalis within the periventricular gray contained 5-HT perikarya while in mesencephalic levels the remainder of raphe dorsalis and the nucleus centralis superioralis up to the collicular junction possessed 5-HT neurons. Additionally, numerous 5-HT perikarya were observed in other brain stem nuclei, including many of the reticular formation. Such cells were seen within the parvocellular and magnocellular portions of the 1) lateral reticular nucleus, 2) nucleus reticularis gigantocellularis, 3) nucleus reticularis pontis oralis, 4) nucleus tegmenti pedunculopontinus-subnucleus dissipatus and within other areas of the brain stem (e.g. medial lemniscus, trapezoid body).

Serotonin histofluorescence also was seen within non-varicose ascending serotonin pathways and could be traced through the brain stem. The 5-HT bundles at mesencephalic levels appeared in a juxtamidline position within the mesencephalic tegmentum, medial to the red nucleus and dorsomedial to the medial tip of the substantia nigra. At these levels 5-HT bundles appeared as a prominent group of fibers which often intermingled with catecholaminergic fibers presumably arising from the substantia nigra. The ascension of these pathways into the hypothalamus currently is being traced. It would appear that the mixing of serotonergic and catecholaminergic fibers could make the specific neurochemical stimulation and/or ablation of these ascending pathways most difficult in the brain stem.

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672 CATECHOLAMINERGIC TERMINALS IN THE HEPATIC ACINUS AND LANGERHANS ISLET OF THE PANCREAS OF RHESUS MONKEYS, AND EFFECTS OF COELIAC ARTERIAL INJECTION OF 6-, AND 5-HYDROXYDOPAMINE. Hideo Uno* (SPON: M. Sato). Lab. Pathol., Oregon Regional Primate Res. Ctr., Beaverton, OR. 97005

There is no convincing evidence in the literature of catecholaminergic nerve innervation of the hepatic parenchymal cells. We used the method of catecholamine histofluorescence (Falack & Hillarp) to demonstrate the nerve terminals containing fluorescent catecholamine in the hepatic acinus of rhesus monkeys (Macaca mulatta). The varicose terminals contact closely with the hepatic cell cords and run through the peripheral to the central zone of the entire acinal structure. In nontreated control animals, these fibers are found sporadically in the hepatic acini. After treatment with monoamine oxidase inhibitor "Nialamide," the number of intensely fluorescent fibers increased in each acinus in many areas of the lobes.

Catecholamine-containing terminals in the pancreatic islets of mammals have been reported by several authors. In normal rhesus monkeys, the varicose terminals were found mostly along the peri- and intra-acinal capillaries. After treatment with Nialamide, the intensity of fluorescence in these fibers also increased.

Ultrastructurally, the terminals containing many small and a few large dense-cored vesicles were found in both the perisinusoidal space of Disse and in close contact with the hepatocytes. The terminals in the pancreatic islets were located in perivascular and interacinal spaces.

Nine animals were divided into three groups and injected with either 6-hydroxydopamine (6-OHDA), 5-hydroxydopamine (5-OHDA), or saline HCl solution into the coeliac artery following ligation of the splenic artery. Ten mg of each drug, dissolved in 5 ml saline HCl solution, were used for the injection. Thirty minutes after injection, the animals were sacrificed. The fluorescence of the terminals in the hepatic acini and pancreatic islets was reduced by 5-OHDA, and 6-OHDA caused complete disappearance. Ultrastructurally, granular density of the dense-cored vesicles in the terminals of both the hepatic acini and pancreatic islets was significantly increased by 5-OHDA, whereas 6-OHDA induced marked swelling of many vesicles in which the granules either disappeared or were irregularly condensed. The terminal axons containing many small agranular vesicles and a few large dense-cored vesicles (comparable to cholinergic terminals) were also found near the hepatic cells and in the islets, but they remained intact despite the drug treatment. These results indicate that the hepatic acinus and pancreatic islets of rhesus monkeys are innervated with both catecholaminergic (adrenergic) and cholinergic nerves.

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673 LOCUS COERULEUS TO DORSAL RAPHE CONNECTIONS: ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL STUDIES. Clint D. Anderson,* Daniel A. Pasquier,* William B. Forbes and Peter J. Morgane (SPON: Robert D. Hall). Providence College, Providence, RI 02918 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The effect on Dorsal Raphe (DR) single neuron firing has been explored following electrical stimulation to the Locus Coeruleus (LC) in a series of acute rats. Under chloral hydrate anesthesia, glass recording microelectrodes and side-by-side stainless steel stimulating electrodes were inserted in the target sites. Stimulus trains of .5 to 7 sec at 5 to 10 Hz were administered with currents between 100 and 500 uA. In six preparations with histologically verified placements, inhibitory effects were manifested in three instances as 25 to 50% slowing of unit firing rate for periods of 3 to 5 sec. The inhibition often was clearest upon the first stimulation and was unreliable with repeated stimulation. Excitation was never observed. Fast, steadily firing cells (rates up to 30/sec) in the central gray area near the dorsal raphe often responded by becoming oscillatory in their firing rates just after the stimulation of LC. In parallel studies we have used retrograde transport techniques to seek morphological evidence for LC to DR connections. After horseradish peroxidase injections were placed precisely in the DR, labeled cells were only sparsely observed in the LC, although positive transport to other nuclei support the validity of our technique. It is possible that LC to DR connections are primarily indirect. Investigations are continuing for the purpose of clarifying these relationships. (Supported by NSF grant BNS 74-02620).

674 EPINEPHRINE IN THE MONKEY BRAIN. E.T. Angelakos, J.D. Irvin, S. Jacobs and I. Mohler*. Hahnemann Med. Col., Philadelphia, PA. 19102 and Univ. of Calif., Santa Barbara, CA. 93107.

The distribution of norepinephrine (NE) and epinephrine (EPI) were measured in various regions of the squirrel (S. Sciureus) and macaque (M. Mulatta) monkey brains. Significant amounts of EPI were found in the medulla, pons, hypothalamus, olfactory bulb and cingulate cortex but not in the other regions of the cortex (occipital, frontal, parietal). EPI concentrations were highest in the hypothalamus. However, the highest EPI/NE ratios were found in the olfactory bulb and the cingulate cortex. Studies with the fluorescence histochemical technique in these species indicated catecholamine containing neurons in the medulla and pons but not in the hypothalamus. Occasionally catecholamine containing cells (neurons?) were found in the olfactory bulb and cingulate cortex which could account for the relatively high concentration of EPI in these regions. It is tentatively concluded that EPI containing neurons in the medulla and pons send projections rostrally with terminals in the hypothalamus and possibly in the cingulate cortex. This system appears to be more prominent in the subhuman primate than in the phylogenetically lower species. (Supported by NIH Hl 13008).

675 THE ROLE OF SEROTONIN (5HT) RELEASE IN THE BEHAVIOURAL EFFECTS OF PARAMETHOXYPHENYLETHYLAMINE (PMPEA) IN MICE. R. Ashkenazi and M. Weinstock* (Supported partly by NIH Grant NS 11255). Dept. Physiol., Hebrew Univ.-Hadassah Med. Sch., Jerusalem, and Dept. of Physiol.-Pharm., Sackler Med. Sch., Tel-Aviv, Israel.

Paramethyoxyphenylethylamine (PMPEA) administration to rats and mice results in a transient behavioural response characterized by side to side head movements, hind legs spread and tail erection, tremor and hyper-excitability to tactile stimuli. This response is abolished by pretreatment with reserpine, which depletes monoamines in the central nervous system, and is intensified by monoamine oxidase inhibitors like pargyline. This indicates that monoaminergic systemsare involved in the behavioural response caused by PMPEA. Blocking 5HT synthesis by p-chlorophenylalanine (PCPA) caused a marked decrease in the behavioural response to PMPEA. Methysergide, a known 5HT antagonist in the spinal cord, had no effect on head movements but blocked the hind legs spread and the tremor and decreased the sensitivity to tactile stimuli. Head movements alone could be blocked by low doses of Haloperidol, but at the same time this drug intensified the hind-leg spread. Propranolol, a β-adrenoceptor antagonist that can also antagonize responses to 5HT, had a small effect on head movements but markedly decreased the hind legs spread. These experiments indicate that PMPEA like its parent compound phenylethylamine (PEA) is an indirect sympathomimetic amine, and the behavioural response to this compound is due to its ability to release monoamines in the CNS. Unlike PEA, which releases only catecholamines, PMPEA releases 5HT as well, hence the difference in the behavioural responses to these two compounds. Our experiments also indicate that although catecholamines are involved in the behavioural response 5HT release is necessary to initiate it.

676 DISCRETE RAPHE LESIONS: EFFECTS ON ACTIVITY, AMPHETAMINE STEREOTYPY AND STRAIGHT ALLEY ACQUISITION AND EXTINCTION. Karen E. Asin, David Wirtshafter* and Ernest W. Kent. Dept. Psychol., Univ. Il. at Chicago Circle, Chicago, Il. 60680.

Rats were prepared with lesions of the medial (MR), dorsal (DR), or combined medial and dorsal (MDR) raphe nuclei. Relative to sham operated controls, MR and MDR animals displayed a pronounced hyperactivity in the open field, whereas DR subjects exhibited a smaller, but still significant, enhancement of activity. Influences of the raphe nuclei on stereotypy were assessed following i.p. injection of 10 mg/kg d-amphetamine. The suppression of locomotion seen in control and DR animals was greatly attenuated in MDR and MR animals; members of these groups often engaged in brief bouts of running between episodes of intense gnawing. The licking and gnawing behaviors seen in DR subjects appeared, in most cases, more vigorous than those demonstrated by controls. These observations suggest that although the MR is not essential for the expression of certain components of amphetamine stereotypy, it does appear to contribute to the locomotor depression produced by high doses of amphetamine.

Subsequently, rats were maintained at 85% of their body weight and run on a straight alley task for food reward. Both MR and MDR animals showed slower running speeds during acquisition than members of the other two groups. During extinction, MR and MDR animals failed to decrease their running speeds as rapidly as control and DR subjects. The performance of DR rats was indistinguishable from that of controls on all aspects of the task. The combination of impaired acquisition and increased resistance to extinction in the straight alley appears unique to destruction of the medial raphe and has not been reported following damage to limbic structures.

677 EXCITATION OF LATERAL SEPTAL NEURONES FOLLOWING STIMULATION OF THE A_{10} DOPAMINERGIC SYSTEM. S.Y. Assaf*, J.J. Miller, Dept. Physiology, Univ. British Columbia, Vancouver, B.C. Canada. V6T 1W5.

Previous studies have demonstrated that the lateral septal region contains relatively large amounts of dopamine (DA) and a high density of dopaminergic nerve terminals. Lesions of the ventral mesencephalic tegmentum or medial forebrain bundle have been shown to result in a complete loss of septal DA fibers. These data suggest that a pathway originating in mesencephalic DA containing neurones innervates the lateral septal region. The present experiments were undertaken to further delineate the cells of origin of this projection system and to determine the effect which it exerts on extracellularly recorded neuronal activity in the septal area of the rat. Unilateral injections of horseradish peroxidase into the lateral septal region resulted in labelling of neurones confined mainly to the A_{10} mesencephalic dopaminergic cell group. Single pulse stimulation of the A_{10} region evoked responses consisting of an activation or activation-inhibition sequence in identified lateral septal neurones. The latency to activation was 8-10 msec and in spontaneously firing cells the duration of inhibition which follows the initial activation ranged between 30-110 msec. The synaptically evoked excitation was reversibly blocked by injections of the dopamine antagonists haloperidol and flupenthixol. In 6-OHDA treated preparations in which the DA content of the septal area was depleted by 80-95%, A_{10} stimulation was ineffective in eliciting responses from lateral septal neurones. These data provide morphological and electrophysiological evidence for an anatomical projection from the A_{10} mesencephalic cell group to the lateral septal region and indicate that DA may be an excitatory transmitter in this system.

(Supported by the Medical Research Council of Canada).

678 A SEROTONIN-CONTAINING NERVE CELL GROUP IN RAT HYPOTHALAMUS. Alain Beaudet* and Laurent Descarries (SPON: S. Rossignol). Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec.

Following prolonged ventricular perfusions with 10^{-5} or 10^{-4} M tritiated serotonin (5-HT-^{3}H), intensely labeled nerve cell bodies were detected in radioautographs from the dorso-medial hypothalamus of adult male albino rats pretreated with a monoamine oxidase inhibitor. These reactive perikarya were loosely grouped amidst unlabeled neurons, within pars ventralis of the nucleus dorso-medialis. In the light microscope, the labeled cells did not appear morphologically distinct from their unlabeled congeners, and corresponded to the small (mean diameter: 10 µm) roughly spherical neurons characteristic of this nucleus. Comparable numbers of reactive perikarya were consistently found after 10^{-5} and 10^{-4} M 5-HT-^{3}H. In order to verify the specificity of this radioautographic reaction, two types of control experiments were carried out: 1) ventricular perfusions with 10^{-5} or 10^{-4} M tritiated norepinephrine (NA-^{3}H), and 2) simultaneous administration of 10^{-4} M 5-HT-^{3}H and 10^{-3} M non-radioactive norepinephrine. Whereas no labeled cells were detected in the dorso-medial nucleus after intraventricular NA-^{3}H, there was no apparent diminution in the number and intensity of the nerve cell body reactions induced by 5-HT-^{3}H in the presence of cold norepinephrine. These data suggest the existence of a previously undescribed group of presumptive serotoninergic neurons in the dorso-medial nucleus of hypothalamus. Such intrinsic neurons could contribute to the serotonin innervation of several hypothalamic nuclei and/or the median eminence, and thus play a role in neuroendocrine regulations. It might be appropriate to designate this cell group as B-10, in accordance with the nomenclature of Dahlström and Fuxe. (Supported by a fellowship and grant MT-3544 from the Medical Research Council of Canada).

679 EFFECTS OF PIRIBEDIL ON NEURONS IN THE CAUDATE NUCLEUS AND CEREBRAL CORTEX. B. Bioulac*, A. Ferron* and E. Puil (SPON: J. de Champlain), Dép. de Neurophysiologie, Univ. de Bordeaux, Bordeaux, France and Centre de Recherche en Sciences Neurologiques, Univ. de Montréal, Montréal, P.Q.

Agents which have been found to be useful in the treatment of Parkinson's disease appear to cause their therapeutic effect by a cholinolytic action or by stimulating receptors for dopamine, possibly at the level of the basal ganglia. As an antiparkinsonian drug, piribedil (1-(3,4-methylenedioxybenzyl)-4-(2-pyrimidyl)-piperazine) might be expected to act in either way (or both). The effects of microiontophoretically administered piribedil (Servier; methane sulfonate, 0.1M, pH 4.0) were studied during extracellular recording from neurons in anesthetized cats and rats or in decerebrate cats. The spontaneous firing of cortical cells which when tested were found to be excited with acetylcholine, was strongly reduced for periods of about 6 min. by brief (30s) applications of piribedil. Neurons not discharging spontaneously but excited with glutamate, were also depressed by piribedil but this depression outlasted the period of its application by only about 1-2 min. The same applications of piribedil and dopamine to neurons in the caudate produced comparable inhibition of firing although recovery was more rapid, usually requiring less than 1 min. In all cases, the effect of this agent was similar to that of dopamine. Piribedil (20-100 nA) appeared to be more potent than dopamine (50-150 nA) in the cerebral cortex while the compounds seemed to be equipotent in the caudate. The similarity of the effects of piribedil to those of dopamine in both structures suggests that this antiparkinsonian agent may exert its therapeutic action by activating inhibitory receptors for dopamine in the central nervous system.

680 DYNAMICS AND DISPOSITION OF REDUCED PTERINS IN THE RAT BRAIN. Wilson P. Bullard*, Arnold J. Mandell and Patrick V. Russo*. Dept. Psychiat., Sch. Med., UCSD, La Jolla, CA 92093

We have obtained a profile of the pharmacology and regional and subcellular distribution of endogenous reduced pterins (PH_4) capable of subserving tyrosine hydroxylase (TOH) and tryptophan hydroxylase (TpOH) in the rat brain. Across ten regions there is a significant correlation between PH_4 activity and TOH activity, and the correlation improves with an *ad hoc* proportional correction of total PH_4 for TpOH-linked PH_4. That is, a correlation with TpOH activity appears likely, but is not as yet demonstrable. Correlation of PH_4 with regional quinoid dihydropterin reductase (QPH_2R) activity is not observed. Subcellular distribution of PH_4 in the striatum indicates about 45% to be associated with an enriched synaptosomal fraction. This estimation is approximate, however, due to differential rates of PH_4 decay in the various subcellular fractions. Comparison of endogenous PH_4 levels and synaptosomal synthesis of dopamine and serotonin *in vitro* suggests that more than a stoichiometric amount of PH_4 is consumed, and must therefore be regenerated, presumably *via* QPH_2R. Administration of d-amphetamine (5 mg/kg i.p., 2 hrs) or reserpine (2.5 mg/kg i.p., 24 hours) elicits a 25% decrease ($p < 0.01$) or a 40% increase ($p < 0.02$) respectively in our measure of endogenous striatal PH_4. Haloperidol and chlorpromazine fail to elicit any alteration in PH_4 level. The data suggest 1) that a significant portion of rat brain PH_4 is functionally linked to TOH and TpOH, 2) that *in situ* regeneration of PH_4 is operative, 3) that subcellularly, PH_4 is heterogeneously distributed, and 4) that some psychoactive drugs are capable of altering the disposition and/or dynamics of reduced pterins in the brain. This work is supported by USPHS grant DA-00265-04.

681 INVOLVEMENT OF SEROTONIN FIBERS IN L-DOPA STIMULATED MOTOR ACTIVITY FOLLOWING 6-OHDA TREATMENT. M. G. Cantrell*, A. S. Hollister* and G. R. Breese. Depts. Psychiatry and Pharmacology, University of North Carolina, Chapel Hill, N. C. 27514.

In order to define the role of brain monoamine fibers in the stimulation of locomotor activity by L-DOPA, animals with preferential destruction of brain dopamine (DA), norepinephrine (NE), serotonin (5HT), or combinations thereof were tested for their locomotor response to L-DOPA and apomorphine. Animals with 6-OHDA-induced destruction of DA or both catecholamine (CA) fiber systems in brain showed large increases in locomotor stimulation by L-DOPA while animals depleted of NE or 5HT and untreated animals did not. Inhibition of NE synthesis by treatment with U-14,624 did not reduce the heightened activity response to L-DOPA in DA and CA depleted animals. Over a wide variety of treatment paradigms the locomotor activity response to L-DOPA displayed a significant negative correlation to brain concentrations of DA, and brain 5HT levels were positively correlated when DA levels were controlled for. The response to L-DOPA, but not to apomorphine was antagonized in a dose-related fashion by destruction of brain 5HT fibers in DA and CA depleted rats. The accumulation of ^{3}H-DA after ^{3}H-L-DOPA in DA and CA depleted animals was also decreased by destruction of brain 5HT-containing fibers. These findings suggest that (1) destruction of brain DA-containing systems potentiates the locomotor response to L-DOPA (2) that the stimulation of motor activity by L-DOPA in DA and CA depleted rats is not dependent upon the synthesis of NE and (3) that brain 5HT-containing fibers contribute to the decarboxylation of L-DOPA to DA in animals depleted of DA or both catecholamines. These findings suggest that 5HT-containing fibers play an important role in the efficacy of L-DOPA therapy in Parkinson's disease. (Supported by USPHS Grants MH-16522, HD-03110, ES-1-P01-ES01-104-01 and the PMA Foundation, Inc.)

682 EFFECTS OF UNILATERAL NOREPINEPHRINE AND DOPAMINE DEPLETING LESIONS ON RESPONSE RATES GENERATED BY BRAIN STIMULATION REWARD. Robert J. Carey and John B. Murphy*. VA Hospital, Syracuse, NY 13210

The effects on brain stimulation reward of unilateral norepinephrine or dopamine depleting lesions were evaluated in rats having functional bilateral medial forebrain bundle electrode placements. Preoperatively, stable 15 min. baseline response rates and rate-intensity functions were determined for both electrode sites in each rat. This paradigm of producing a unilateral brain lesion in animals with functional bilateral electrode placements permitted a within animal comparison of lesion versus non-lesion electrode sites. Postoperatively, response rates for brain stimulation reward were tested on the lesion and non-lesion halfs of the brain for up to 4-6 weeks and rate intensity functions were again determined. Lesions which produced a significant unilateral decrease in striatal-cortical norepinephrine facilitated response rates on the lesion side whereas dopamine depleting lesions decreased response rates on the lesion side. Response rates on the intact sides tended to remain unchanged and lesions which did not decrease norepinephrine or dopamine generally had no effect or response rates obtained from electrodes on either the lesion or non-lesion sides of the brain.

683 CHARACTERIZATION OF CATECHOLAMINE RECEPTORS ON NORADRENERGIC NEURONS OF THE LOCUS COERULEUS. Jesse M. Cedarbaum and George K. Aghajanian. Depts. of Psychiat. and Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508

Several studies have demonstrated the presence of catecholamine (CA)-containing nerve terminals in rat locus coeruleus (LC). In addition, it has been shown that the noradrenergic neurons of the LC are inhibited by microiontophoretic norepinephrine (NE), epinephrine (E), the α-adrenergic agonist clonidine (CLON), or the β-agonist isoproterenol (ISO). This inhibition is specifically blocked by the α-antagonist piperoxane but not by the β-antagonist sotalol. Furthermore, low i.v. doses of piperoxane markedly activate LC neurons, possibly through blockade of a tonic, inhibitory-CA input. We now further characterize the CA receptors on LC neurons with respect to the actions of other drugs which act at CA synapses. Experiments were done in chloral hydrate anesthetized male albino rats, using microiontophoretic and single-unit recording techniques. In addition to being inhibited by iontophoretic NE,E,CLON and ISO, LC units were inhibited by systemic injections of d-amphetamine and desmethylimipramine. These inhibitions were rapidly reversed by low i.v. doses of piperoxane. Iontophoretic dopamine (DA) but not the DA agonist apomorphine also promptly inhibited LC cells; d-amphetamine when applied by iontophoresis also resulted in decreased cell activity, but only after 3-5 min, suggesting an indirect mode of action. The potent DA antagonist trifluoperazine failed to block the inhibition of LC cells by either DA or NE even when used in amounts much greater than required to block the inhibition of dopaminergic neurons of the substantia nigra by DA (Aghajanian & Bunney, in prep). Taken together, these results suggest that central NE neurons receive an inhibitory CA input; the receptors mediating this inhibition have pharmacological properties resembling those of peripheral presynaptic α-receptors, and are distinct from central DA and β-receptors. (USPHS Grant MH 17871 and the State of Connecticut).

684 RECOVERY OF SUBSTANTIA NIGRA SELF-STIMULATION AFTER UNILATERAL 6-HYDROXYDOPAMINE LESIONS OF THE NIGROSTRIATAL PATHWAY. R. M. Clavier and H. C. Fibiger. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, University of British Columbia, Vancouver, B.C.,V6T 1W5,Canada.

These experiments investigated the role of the dopamine (DA) containing nigrostriatal pathway (NSP) in intracranial self-stimulation (ICSS) from the substantia nigra in rats. Nigral ICSS rates, in 15 min. sessions, were compared before and after ipsilateral (n=14) or contralateral (n=16) 6-hydroxydopamine lesions of the NSP. Also studied were the effects of these lesions on: 1) prolonged (1 hr) ICSS performance; and 2) the ability of d-amphetamine (1 mg/kg, ip) to influence this ICSS. Striatal DA was depleted by an average of 97.5% in all subjects. 24 hrs after the lesions, ICSS was reduced to less than 10% of prelesion values. Both groups recovered, however, and by the 8th-10th postlesion day ICSS reached 100% of prelesion rates. ICSS in the 1 hr sessions was reduced slightly, and comparably, in both groups after the lesions. D-amphetamine elevated ICSS reliably in both groups prior to the lesions and in the contralateral group after the lesions. However, there were significantly fewer cases of increased ICSS in the group with ipsilateral NSP lesions. Thus, the NSP seems to be involved in the effects of d-amphetamine on nigral ICSS, and in the ability of rats to perform the operant tasks set by the ICSS paradigm. While these data do not deny a possible contribution of the NSP to the reinforcing properties of nigral ICSS, this contribution is clearly not essential for the occurrence of the behavior. (Supported by the Medical Research Council)

685 CARBONYL BINDING REAGENTS MARKEDLY AUGMENT THE RELEASE OF ^{3}H-DOPAMINE EVOKED BY POTASSIUM IONS IN SLICES OF RAT STRIATUM. Gerald Cohen & Dorothy Dembiec.* Dept. Neurology, Mount Sinai School of Medicine, Fifth Avenue and 100th Street, New York, N.Y. 10029.

We had previously reported (Dembiec & Cohen, Biochemical Pharmacol., in press) that three carbonyl-binding reagents, namely bisulfite, phenylhydrazine and hydroxylamine, augmented the release of ^{3}H-norepinephrine (^{3}H-NE) by 160-250% from sliced mouse heart during stimulation with potassium ions. Experiments with cocaine or with 6-hydroxydopamine showed that the mechanism for this effect was not blockade of reuptake of released ^{3}H-NE and that the source of the increased release of ^{3}H-NE was the sympathetic nerve terminals of the heart. In the current study, a number of other tissues were surveyed for the effects of the carbonyl reagents and were found to behave differently from the heart. In the submaxillary gland, for example, the carbonyl reagents did not evoke increased release of ^{3}H-NE; in fact, hydroxylamine actually decreased the release of ^{3}H. The occipital cortex, an area rich in NE terminals, was compared to the striatum, an area rich in dopamine (DA) terminals. In the occipital cortex prelabelled with ^{3}H-NE, 1 mM bisulfite and 1 mM hydroxylamine increased the K^{+}(15mM)-stimulated release of ^{3}H by 37% and 48%, respectively, while 1 mM phenylhydrazine did not exert a significant effect. In the striatum prelabelled with ^{3}H-DA, the same concentrations of carbonyl reagents (1 mM) elevated the release of ^{3}H-DA in the range 180-360%. Thus, the striatum and heart appear to be extremely sensitive to the action of the carbonyl reagents. The mechanism of this action has not been clarified, but it may involve the presence of regulatory aldehydes or ketones in synaptic membranes.

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686 ENTORHINAL CORTEX (EC): CATECHOLAMINE FLUORESCENCE AND NISSL STAINING OF IDENTICAL SECTIONS. Timothy J. Collier* and Aryeh Routtenberg (SPON: J. J. Pysh), Cresap Neuroscience Laboratory, Northwestern University, Evanston, Illinois, 60201.

Recent histochemical fluorescence data has described dopamine containing fibers and presumptive terminals in four cortical areas, including EC (Lindvall, et al. Br. Res. 81 (1974) 325). Using glyoxylic acid fluorescence methods, this region of posterior temporal cortex was found to contain dopamine innervation arranged in distinct islands in the second and third cortical layers. In our examination of this area, vibratome sections of EC were examined for catecholamine fluorescence, and then stained with thionin for cell bodies. Four findings were particularly salient: 1) The existence of the previously described dopamine islands of EC using the glyoxylic acid method can be confirmed using the paraformaldehyde method of Hökfelt and Ljungdahl (Histochemie 29 (1972) 325). 2) The islands of dopamine innervation in EC are present only at anterior levels; a rich catecholamine innervation, without islands, is present at other levels. 3) A potential cellular substrate for the islands of dopamine innervation in EC can be observed by thionin counterstaining of the same tissue used for fluorescence examination. Islands of small darkly-staining cells and larger light-staining cells are distributed through the second and third cortical layers and appear to be closely associated with the islands of dopamine fluorescence. 4) Electrolytic lesions of the A10 dopamine containing cell group indicate ipsilateral A10 projection to EC as one source of the dopamine islands. Since EC supports self-stimulation and is associated with memory consolidation, the dopamine islands of EC may be involved in these functions. Supported by The Alfred P. Sloan Foundation, NS 10768, MH 25281 and BMS 19481 to A. R.

687 DESCENDING MONOAMINERGIC PATHWAYS: A STUDY UTILIZING SPINAL CORD LIGATION. Keith A. Crutcher and W.G. Bingham. Depts. of Anatomy and Surgery (Neurosurgery), College of Medicine, Ohio State Univ., Columbus, Ohio 43201.

The origin of descending monoamine-containing fibers has been investigated with a series of ligations at various thoracic levels in the spinal cord of the rhesus monkey. Fluorescence histochemistry and electron microscopy were used to determine alterations in neuronal perikarya in the brainstem. Ligation of the spinal cord produces a marked increase in the fluorescence intensity of neurons within the ventrolateral medulla near the lateral reticular nucleus and a less pronounced increase in neurons of the locus coeruleus. In addition there is an increase in axonal fluorescence within a number of areas in the lateral and ventral funiculi of the spinal cord above the ligation.

Ultrastructurally, a number of cell bodies within the locus coeruleus exhibit increased cytoplasmic density accompanied by an accumulation of mitochondria. Thin sections of the lateral and ventral funiculi above the level of the ligation reveal numerous profiles of axons with accumulations of organelles including, in some fibers, dense core vesicles. A large number of profiles also contain cellular elements which appear to be phagocytic.

Although it is difficult to directly compare the two techniques, the results support the possibility that the increase in neuronal and axonal fluorescence correlates with the dark reaction and organelle accumulation seen with the electron microscope.

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688 ORIGIN OF URINARY MHPG-GLUCURONIDE VS. MHPG-SULFATE. H. Dekirmenjian, J. M. Davis and J. W. Maas. Ill. State Psychiat. Instit., Chicago, Ill. 60612 and Dept. of Psychiat., Yale Univ. Sch. Med., New Haven, Conn. 06510

It is generally accepted that brain norepinephrine (NE) is primarily metabolized to 3-methoxy-4-hydroxy phenethylene glycol (MHPG), and that an appreciable fraction of urinary MHPG has had its origin in the turnover of brain NE pools. In human urine, MHPG is excreted as the conjugates of either glucuronide or sulfate. Recently, it has been suggested that urinary MHPG-sulfate is a better index of brain NE metabolism than total MHPG (Bond and Howlett, Biochem. Med. 10:219, 1974). To study the relative contribution of brain NE metabolism to urinary MHPG-sulfate, rats and rabbits were injected I.P. by H^3-normetanephrine (NM) or H^3-MHPG and 24 hr. urines were collected and MHPG-glucuronide and MHPG-sulfate separated on a DEAE Sephadex column. Both endogenous and H^3-labeled metabolites were assayed. H^3-NM and H^3-MHPG do not pass the blood brain barrier, thus urinary levels of H^3-MHPG-glucuronide and H^3-MHPG-sulfate would have had their origin in the metabolism of peripheral pools, while the levels of endogenous MHPG-glucuronide and MHPG-sulfate would have had their origin in the NE metabolism of both the periphery and the brain. Results obtained from these experiments show: 1) that in the CNS as well as in the periphery NM is only metabolized to MHPG; 2) R.S.A. of H^3 labeled to endogenous MHPG-glucuronide vs. MHPG-sulfate is 2:1 in H^3-NM infusion; 3) while in H^3-MHPG infusion R.S.A. of MHPG-glucuronide vs. MHPG-sulfate is 1:1; 4) finally, in human male controls vs. female controls the ratio of MHPG-glucuronide vs. MHPG-sulfate is the same, although male controls excreted 400 ug/24 hr. more total MHPG than female controls. The above data in conjunction with data from human brains, where all of the MHPG is in the free form, suggests that, in the human, urinary levels of MHPG-sulfate are not better indicators of brain NE metabolism.

689 SEROTONINERGIC INNERVATION OF BLOOD VESSELS IN PRIMATE BRAIN STEM? Vincenzo Di Carlo. Indiana Univ. Sch. of Med., Gary, Ind. 46408

Using the technique of Falck and Hillarp, yellow-fluorescing fibers and varicosities were detected within the wall of microvessels in the brain stem of a primate, the squirrel monkey. The wave length of the emitted fluorescence, the partial fading on exposure to UV light of the fluorophor contained in the fibers and the frequent close relationship of such fibers with serotonin-containing nerve cell bodies support the identification of at least some of such fibers and varicosities as serotoninergic in nature. Such fibers are most frequently present together with green-fluorescing fibers, which are interpreted as being catecholaminergic in nature; however, they are sometimes found to be the only fluorescing fibers within microvessels, especially in the pontine raphé. No serotoninergic fibers were found to be present in the basilar artery or its main branches. The tentative interpretation of these findings is that brain stem serotoninergic neurons may innervate microvessels within the brain stem itself. These observations, which deserve verification by other techniques, raise the question whether also some of the catecholaminergic fibers innervating blood vessels might originate from brain neurons. Moreover, serotoninergic neurons embracing blood microvessels with their yellow-fluorescing processes were also observed in the pontine raphé. This finding appears to be in agreement with an observation by Scheibel and Scheibel on Golgi-stained material from rodents.

690 AGE-RELATED RECOVERY OF REGIONAL 5 AND 20 MINUTE UPTAKE VALUES OF ^{3}H-NORADRENALINE AND ^{3}H-DOPAMINE IN RAT BRAIN AFTER RESERPINE. Jean DiRaddo, Dept. of Psychology, Univ. of Rochester, Rochester, N.Y. 14627.

Long-Evans hooded rats (aged 10, 28 days and 10 wks) were injected with 5 mg/kg reserpine (i.p.) or the drug vehicle and sacrificed 2, 24, or 72 hrs later. Cortical and hypothalamic slices were incubated with 10^{-7}M ^{3}H-noradrenaline (NA) and striatal slices were incubated with 10^{-7}M ^{3}H-dopamine (DA) for 5 or 20 min. In 10 day-old controls, 5 min uptake in striatal samples increased with age so the 72 hr (13 day) value was double that seen at any other time or age. No other age-related differences in 5 min uptake were observed. In the cortex 20 min uptake at 10 and 28 days was 1-1/2 times that seen at 10 wks and in the striatum 20 min uptake at 10 days was twice that seen at 28 days and 10 wks. Transmitter uptake may serve a more critical function at ages when storage pools of transmitters have not yet reached mature levels. In reserpinized Ss there were age-specific variations in recovery of 5 min uptake but all 3 regions showed the same pattern: at 2 hrs, 10 day Ss showed the least depletion; at 24 hrs, 28 day Ss showed the greatest recovery; and at all times 10 wk Ss showed less recovery than 28 day Ss. All ages showed a similar depletion of 20 min uptake at 2 hrs in cortex and striatum. As with 5 min uptake, 28 day Ss showed the greatest recovery at 24 hrs in all regions. The marked effect of reserpine on the 5 min uptake supports the suggestion that this short incubation reflects early accumulation of transmitter into vesicles as well as membrane transport (Azzaro & Smith, J. Neurochem., 1975, 24, 811). The early recovery of 28 day Ss relative to the other ages correlates well with the earlier behavioral recovery in response to reserpine at this age. Data from samples incubated with inhibitors of monoamine oxidase and catechol-O-methyltransferase will also be presented.

691 EFFECTS OF LOCUS COERULEUS OR SUBSTANTIA NIGRA LESIONS ON SOCIAL BEHAVIOR IN RAT COLONIES. Michael S. Eison*, Arlene D. Stark*, Gaylord Ellison, and David Masuoka. Dept. Psychol., UCLA and Neuropharmacology Lab., VA Sepulveda, Los Angeles, Ca. 90024.

Rats were observed in an enriched colony environment following RF lesions of Locus Coeruleus (LC), pars compacta of Substantia Nigra (SN) or control electrode placements. LC animals were initially inactive, poorly coordinated, and stayed in the burrows. They progressively fell in dominance. SN rats were initially hyperactive and had minimal motor disturbances. They became progressively more violent and persistent in their attacks. Unlike other hyperagressive rats seen in previous colony studies (5,6 DHT), SN animals failed to respond to social cues that typically end aggressive encounters and they were instrumental in the killing of a number of LC animals. Whereas the SN rats self-groomed and avoided social interactions, the LC animals mounted and engaged in social grooming. The behavioral syndrome following LC lesions resembles that found after intraventricular 6-OHDA administration but SN animals are distinctively different from other rats with monoamine alterations.

692 DOPAMINE INNERVATION OF SOME BASAL FOREBRAIN AREAS IN THE RAT. James H. Fallon and Robert Y. Moore. Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

The projection of the substantia nigra and ventral tegmental area to the neostriatum, olfactory tubercle, nucleus accumbens, and some cortical areas is well known. In the present study the dopamine innervation of basal forebrain areas in the temporal lobe of the rat was analyzed using the glyoxylic acid fluorescence method, the autoradiographic tracing method, the horseradish peroxidase-retrograde transport method, and the isotopic enzymatic assay of Coyle and Henry (1972).

In glyoxylic acid histochemical material, dopaminergic (DA) fibers arising from the pars compacta of the substantia nigra (SN) course anteromedially through the ventral tegmental area (VTA). The SN and VTA both contribute DA fibers ascending in the medial forebrain bundle. DA fibers, en route to basal forebrain areas, leave the medial forebrain bundle and enter the ansa peduncularis-ventral amygdaloid bundle and distribute terminals to the amygdaloid complex, especially the central, lateral basal, and posterior lateral nuclei and intercalated masses. DA fibers circumventing or passing through the amygdala distribute moderately dense terminals in the claustrum and medial piriform cortex. DA fibers also terminate in clusters in layers II and III of ventral entorhinal cortex.

Lesions of the SN-VTA region result in a dramatic reduction of the fine, smooth fluorescent DA axons in the basal forebrain region. This corresponds to an 80% decrease of DA content ipsilaterally in this region, as determined from the biochemical assay material.

The histochemical and biochemical data correlate well with autoradiographic studies on the SN and VTA projections and studies with horseradish peroxidase-retrograde transport from basal forebrain areas to the SN and VTA, indicating that both the SN and VTA contribute axons to the basal forebrain areas in the temporal lobe. (Supported by NIH grant NS-12080).

693 RAPHE PROJECTIONS TO THE SUBSTANTIA NIGRA: A POSSIBLE MECHANISM FOR INTERACTION BETWEEN DOPAMINERGIC AND SEROTONERGIC SYSTEMS. H. C. Fibiger and J. J. Miller. Dept. Psychiatry and Physiology, University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

There is increasing evidence for interactions between dopaminergic (DA) and serotonergic (5HT) systems in the central nervous system. Thus, lesions of 5HT systems have been shown to influence behaviors which are thought to have a dopaminergic involvement. One mechanism by which this interaction could take place is through DA and 5HT afferents to the neostriatum (Miller et al., Brain Research 97: 133, 1975). Alternatively, since relatively high levels of 5HT have been found in the region of the substantia nigra (SN), a 5HT fiber system may directly innervate DA neurons in this region. In order to examine the possibility of a direct pathway from the raphe 5HT system to the SN, experiments were conducted to determine (1) the cells of origin and terminal projections of the pathway using retrograde/orthograde tracing techniques and (2) the effect of raphe stimulation on extracellularly recorded unit activity in the SN of the rat. Following unilateral injections of horseradish peroxidase into the region of the SN, labelled neurons were identified in the dorsal raphe nucleus (DRN). Injection of H^3-leucine into the DRN nucleus resulted in a significant autoradiographic labelling in the zona compacta region of the SN. Single and repetitive pulse stimulation of the DRN inhibited the discharge of neurons in the SN for periods ranging from 25-140 msec. This inhibitory response was shown to occur on two types of cells recorded in the SN: those antidromically evoked and those exhibiting an activation-inhibition sequence following stimulation of the neostriatum. These data provide evidence for a neuronanatomical substrate for previously reported interactions between DA and 5HT systems.
(Supported by the Medical Research Council)

694 EMERGENCE OF PROFILES CONTAINING SMALL GRANULATED SYNAPTIC VESICLES AFTER CHRONIC PREGANGLIONIC DENERVATION OF A SYMPATHETIC GANGLION. A.J.D. Friesen* and P.L. McGeer. Div. of Neur. Sci., Dept. Psych. Univ. B.C., Vancouver, B. C., Canada, V6T 1W5

During an investigation of ultrastructural changes induced by chronic preganglionic denervation of the rat superior cervical ganglion, we observed an increased number of synaptic profiles and varicosities containing small vesicles with an electron-dense core in decentralized ganglia. Such structures are rarely encountered in normal ganglia, fixed initially with 3.5% glutaraldehyde and 1.5% paraformaldehyde and post-fixed with 1% osmic acid. Since the structural features of these profiles are similar to noradrenergic nerve terminals, we have concluded that they probably represent synaptic nerve endings and varicosities of collateral axons emanating from the principal noradrenergic neuron. They appear to make synaptic contact mainly with dendrites. Ganglia denervated for 14 days contain a greater number of such structures than ganglia denervated for 5 days. There are at least two possible mechanisms which could account for these findings. In response to decentralization, collateral axons of the principal neuron may proliferate resulting in a greater number profiles containing small granulated vesicles. On the other hand, it is known that the noradrenalin content of sympathetic ganglic increases by about 30% after 2 or 3 weeks of preganglionic denervation. This latter change may result in an increase in the noradrenalin content of pre-existing monoaminergic synaptic vesicles which then are more likely to appear granulated after aldehyde and osmic acid fixation. (Supported by the M.R.C.)

695 OPPOSITE BEHAVIORAL EFFECTS PRODUCED BY LOCUS COERULEUS AND MEDIAN RAPHE LESIONS. Mark A. Geyer, David S. Segal, and Arnold J. Mandell. Dept. Psychiat., Sch. Med., UCSD, La Jolla, CA. 92093

In a series of studies in rats we examined the behavioral effects of electrolytic lesions in either the noradrenergic locus coeruleus (A6) (AP -1.0; DV -2.4; ML ± 1.2; Konig and Klippel, The Rat Brain, 1963) or the serotonergic median raphe (B8) (AP 0.1; DV -2.6; ML 0.0) both of which project to the hippocampus and neocortex. Lesions were verified histologically, using the formaldehyde fluorescence method with cryostat sections, and/or enzymatically, using assays of soluble tyrosine hydroxylase or tryptophan hydroxylase. On a variety of behavioral measures qualitatively opposite effects were produced by A6 and B8 lesions. Lesions of B8 markedly increased the magnitude of startle responses to air puff stimuli (30 trials, 30 sec ISI) while lesions of A6 reduced startle response magnitude. These effects parallel the effects of para-chlorophenylalanine (Connor et al., Physiol. Behav. 5:1215, 1970) and alpha-methyl-para-tyrosine (Sorenson and Davis, Pharmacol. Biochem. Behav. 3:325, 1976) on startle responding. When first introduced into behavioral activity chambers rats with B8 lesions were spontaneously hyperactive while rats with A6 lesions were less active than controls. Following adaptation to these chambers, lesioned rats were tested with either 0.5 or 2.5 mg/kg d-amphetamine sulfate, and their locomotor activity was continuously recorded for 4 hours following injection. Relative to sham-lesioned controls, A6-lesioned rats exhibited less locomotor activity; B8-lesioned rats, more locomotor activity with either dose of amphetamine. These results suggest that the noradrenergic and serotonergic neurons innervating the hippocampus and neocortex have mutually opposing effects on behavior and that both pathways are involved in the locomotor stimulant effects of amphetamine. This work is supported by DA-00265-04, USPHS.

696 HISTOFLUORESCENCE OF CATECHOLAMINES AND ULTRASTRUCTURE OF GRANULATED VESICLES IN NORMAL AND DENERVATED RAT CAROTID BODIES AFTER RESERPINE AND 6-HYDROXYDOPAMINE. Arthur Hess, Dept. of Anatomy, Rutgers Medical School-CMDNJ, Piscataway, New Jersey 08854.

Attempts to determine the significance of the granulated vesicles in the glomus cells of the rat carotid body have been undertaken with the use of the electron microscope and the formaldehyde-induced fluorescence (FIF) procedure. Denervated carotid bodies have also been studied to ascertain the significance of a postulated retrograde feedback mechanism by the sensory nerve terminals on to the glomus receptor cells. Chronic denervation, up to 13 months, causes minimal reaction in the glomus cells; the FIF is unaffected. Reserpine causes an almost total depletion of the FIF in the carotid body. This action of reserpine is inhibited somewhat by prior denervation of the carotid body of at least 4 days duration and after afferent nerve terminals have degenerated. The granulated vesicles appear unaffected morphologically despite a depletion of catecholamines by reserpine. 6-hydroxydopamine markedly reduces the FIF of the carotid body and, as seen after initial fixation in osmium tetroxide, is localized within the granulated vesicles. Reserpine administration causes depletion of the 6-hydroxydopamine from the vesicles. 6-hydroxydopamine apparently acts like a false transmitter. (Supported by NIH Research Grant NS 07662.)

697 MIDLINE TEGMENTAL NEURONS WHICH DECREASE FIRING RATE IN DESYNCHRONIZED SLEEP. J. Allan Hobson and Robert W. McCarley. Dept. of Psych. Harvard Medical School, Boston, Mass. 02115

The cellular basis of sleep cycle control has been hypothesized to depend upon reciprocal interaction between two classes of pontine tegmental neuron: executive neurons, selectively active in desynchronized sleep (D), of which the pontine tegmental field giant cell (FTG) is the prototype; and level-setting neurons, selectively inactive in D, which are found in the region of the nucleus locus coeruleus (LC). Reports of similar rate decreases by dorsal raphe nucleus (DRN) neurons suggested that such cells might play the same role in sleep cycle regulation as those of the LC.

We have made 12 stereotaxically guided microelectrode explorations of the anterior pontine tegmentum in each of three cats looking for D-off cells. D-off cells are defined as those with D/W ratios of less than 0.5. Histological localization to the nearest 0.1 mm of 84 recording sites is now complete for one cat. Twelve D-off neurons were found of which 9 were in the three most anterior midline descents. All were in or near three raphe nuclei as follows: DRN (N=6), linearis centralis (N=2), and centralis superior (N=1). These neurons were characterized by low discharge rates in waking (range 0.37 - 9.33 s/s, mean 4.79 s/s), slightly lower rates in S(range 0.27 - 8.00 s/s, mean 3.03 s/s), and markedly lower rates in D (range 0.00 - 0.27 s/s, mean 0.07 s/s). Two striking features characterized these neurons with respect to phasic events of D: 1) many showed abrupt and sustained arrests of discharge before and during the PGO activity that occurs in the transition period prior to D; 2) some units were sporadically reactivated during the intense REM bursts of D. The location and range of rates of these cells is consistent with their being serotonergic and their pattern of discharge suggests a possible permissive role in D sleep generation.

698 THE LOCUS COERULEUS AND FEARFUL BEHAVIOR OF THE NONHUMAN PRIMATE. Y.H. Huang*, D.E.Redmond, Jr., D.R. Snyder, J.W. Maas, Neurobehavioral Laboratory, Department of Psychiatry, Yale University School of Med. New Haven, Ct. 06510.

Electrical stimulation of the locus coeruleus (LC) in 2 chair-restrained Macaca arctoides (stumptail macaques) elicited alerting, turning, scratching and, after cessation of stimulation, open-mouthed pout face accompanied by a "grunt-purr". These behaviors were reproduced in the same animals by presentation of a shock stick or intense human threats suggesting that the LC may be related to the mediation of fearful responses. Further evidence for this view was obtained in 9 monkeys in which the LC was bilaterally destroyed. Eight of these lesioned monkeys no longer displayed fearful responses when passively confronted by humans. These monkeys neither retreated, lipsmacked nor avoided direct eye contact. When offered objects for manipulation, they would reach out of their cage and grasp. However, they did show some avoidance in the presence of a shock stick or active human threat, but the avoidance was markedly attenuated compared to the response of the control monkeys. These behavioral changes were apparent on the first postlesion day, became more pronounced the next 3-5 days and then gradually diminished over the following 10-20 days. One lesioned animal and 4 sham operated controls did not show these behavioral changes. Histological and biochemical analysis indicated that the lesion produced bilateral destruction of the LC and that norepinephrine and its main metabolite, 3-methoxy-4-hydroxy phenylglycol, were almost totally depleted. Thus, both stimulation and lesion studies suggest that the LC may play a role in the mediation of fearful behavior.

699 LOCALIZATION OF MONOAMINES IN THE BRAINSTEM AND DIENCEPHALON OF THE OPOSSUM. A. O. Humbertson, Jr. and K. A. Crutcher. Dept. Anat., Coll. Med., The Ohio State Univ., Columbus, Ohio 43210.

Monoamine-containing neurons and varicosities were identified in the medulla, pons, midbrain and diencephalon of the opossum utilizing the Falck-Hillarp technique. Yellowish-green fluorescence [YGF] was observed in the nucleus alaris and lateral reticular nucleus of the medulla. In the pons YGF was seen in the locus coeruleus, locus coeruleus pars alpha and dorsal sensory nucleus of V. The substantia nigra, area tegmenti ventralis and area profunda tegmenti exhibited YGF. Continuing into the diencephalon YGF was found in area hypothalamica posterior, nucleus paraventricularis hypothalami dorsalis, nucleus dorsalis hypothalami medialis and areas hypothalamica dorsalis and lateralis. Yellowish-orange fluorescence was observed in the nucleus pallidus raphe and nucleus obscurus raphe of the medulla, within the magnus raphae of the pons and in the nucleus linearis, nucleus centralis superior and nucleus dorsalis raphe of the midbrain. Groups of fluorescent varicosities were observed in the following areas of the brainstem and diencephalon: inferior olivary nucleus, lateral reticular nucleus, hypoglossal nucleus, medial nucleus of solitary tract, facial nucleus, trigeminal motor nucleus, nucleus alaris, nucleus parabrachialis medialis, habenular nuclei, nucleus paraventricularis thalami anterior, nucleus paratenialis, area hypothalamica posterior, nucleus paraventricularis hypothalami dorsalis, nucleus dorsalis hypothalami medialis and area hypothalamica lateralis.

The distribution of monoamines in the opossum is similar to other mammals studied to date.

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700 ON THE CENTRAL ANTI-SEROTONINERGIC ACTIONS OF CYPROHEPTADINE AND METHYSERGIDE. J.H. Jacoby and G.F. Bryce*. Department of Pharmacology, New Jersey Medical School (CMDNJ), Newark, N.J. 07103 and Department of Cell Biology, Roche Research Center, Nutley, N.J. 07110.

Cyproheptadine (CPH) and methysergide (METH) are two potent peripheral serotonin (5HT) antagonists which have been frequently utilized with the assumption that they exert a similar action within the CNS. Other putative central 5HT antagonists, such as methiothepin, elevate brain 5-hydroxyindole [5HT and 5-hydroxyindole acetic acid (5HIAA)] levels after administration; a finding compatible with compensatory neurotransmitter synthesis following receptor blockade. To observe the effects of CPH and METH on brain 5-hydroxyindoles, male Sprague-Dawley rats (200-250 gms.) were fasted overnight and injected with a low (2-5 mg/kg) or high (20-25 mg/kg; i.p.) dose of drug and sacrificed 30, 60 or 120 min. later. Neither drug induced an elevation of either 5HT or 5HIAA at any time point; although CPH (25 mg/kg) did elevate brain tryptophan levels throughout. Furthermore, pretreatment of rats with CPH (10 mg/kg) or METH (2 mg/kg) 15 minutes prior to a tryptophan load (50 mg/kg) in animals sacrificed 1 hr. later did not result in a greater elevation of brain 5-hydroxyindoles than observed in saline pretreated animals. These results suggest that CPH and METH may not be effective as central 5HT antagonists, or that central blockade of some 5HT receptors need not result in a compensatory increase of neurotransmitter synthesis.

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701 DOPAMINE SYNTHESIS IN SYNAPTOSOMES: PREFERENTIAL USE OF NEWLY ACCUMULATED PRECURSORS. Gregory Kapatos* and Michael J. Zigmond. (SPON: I. Hanin). University of Pittsburgh, Pittsburgh, PA 15260.

While the synthesis of serotonin, acetylcholine and histamine appear to be regulated in part by the availability of precursor, the synthesis of catecholamines is believed to take place in the presence of excess precursor. However, using synaptosomes prepared from rat striatum, we have made several observations inconsistent with the association of tyrosine hydroxylase (TH) with a large saturating pool of L-tyrosine (Tyr). First, DA synthesis from Tyr is sensitive to alterations in medium Tyr concentration in the range of 0.5-8.0 μM, levels which are well below the estimated concentration of tissue Tyr (0.1 mM) and which do not alter the level of endogenous Tyr in the synaptosome. Second, synthesis obeys Michaelis-Menton kinetics when expressed as a function of the concentration of Tyr in the medium. Third, synaptosomal TH is saturated with Tyr (Km = 0.9 μM) at a medium concentration which is much lower than that needed to saturate the transport mechanism for Tyr entry into the synaptosome (Kt = 15.3 μM). Fourth, synaptosomes are capable of synthesizing DA from L-phenylalanine (Phe) at a Vmax of more than 50% of synthesis rate from Tyr, and with a Km for synthesis from Phe which is identical to that for synthesis from Tyr. Fifth, Tyr is able to inhibit synthesis from Phe (Ki = 0.6 μM) at a concentration which has no discernable effect on endogenous Tyr levels and which does not alter the accumulation of Phe by the synaptosome. These data suggest that Tyr, whether transported from the medium or formed within the synaptosome from exogenous Phe, does not mix with a large pool of endogenous Tyr prior to conversion to dopa. Instead we propose that in synaptosomes, dopamine biosynthesis occurs preferentially from newly accumulated amino acid.

702 DOPAMINE-SENSITIVE ADENYLATE CYCLASE OCCURS IN THE ZONA RETICULATA OF THE SUBSTANTIA NIGRA OF THE RAT BRAIN. John W. Kebabian*, Juan M. Saavedra, and Paulette Setler (SPON: J. Axelrod). NIMH, Bethesda, Md. 20014 and Smith, Kline and French, Philadelphia, Pa. 19101.

The zona reticulata of the substantia nigra of the rat brain is enriched with dendrites of the dopaminergic neurons forming the nigro-neostriatal tract. In this brain region, both the uptake and storage of dopamine, as well as a potassium-stimulated, calcium dependent, release of dopamine have been demonstrated (Nature, 260:260, 1976), thereby suggesting the possibility of dopaminergic transmission in the zona reticulata of the nigra.

Utilizing a microdissection technique, the zona reticulata was isolated from 300 μ frozen sections of rat brain and adenylate cyclase activity was measured in homogenates of this tissue. Dopamine caused a 74% increase (mean ± SEM, n=16) in adenylate cyclase activity. Maximal stimulation of enzyme activity was caused by dopamine, epinine (N-methyl dopamine) and l-norepinephrine; half-maximal stimulation of enzyme activity by these catecholamines was achieved with concentrations of 10 μM, 8 μM and 100 μM respectively. L-isoproterenol was ineffective as an agonist. Chlorpromazine was a competitive dopamine antagonist with an affinity of 67 nM for the dopamine receptor. Thus, the nigral dopamine receptor is similar to the dopamine receptors regulating adenylate cyclase activity in regions of the brain enriched with the terminals of dopaminergic neurons.

Unilateral injection of 6-OH dopamine into nigra, which both destroyed the dopaminergic neurons and depleted the nigral dopamine on the injected side, did not diminish the dopamine-sensitive adenylate cyclase activity. This suggests that this nigral dopamine-receptor is not anatomically coexistent with the dopaminergic neurons.

703 AGE- AND STRAIN-RELATED UPTAKE OF ^{3}H-NORADRENALINE INTO BRAIN REGIONS FROM AUDIOGENIC SENSITIVE OR RESISTANT STRAINS OF MICE. Carol Kellogg and Mark Zuckerman. Dept. Psychol., Univ. Rochester, Rochester, N.Y. 14627

DBA/2J mice are susceptible to audiogenic seizures of full clonic-tonic intensity with maximum sensitivity occurring at 21 days postnatal age. C57BL/6 mice are not normally susceptible to sound-induced seizures. Previous studies have demonstrated differences between the strains in the maturation of central noradrenergic (NA) systems which may bear a relationship to the seizure susceptibility. In the present study, the in-vitro uptake of ^{3}H-NA into minces of brain regions from both strains was studied at 14, 21, and 42 days of age. ^{3}H-NA was added to the samples at a concentration of 10^{-7}M and the samples were incubated for 5 or 20 min. Results demonstrated that in the resistant strain there was a constant accumulation of ^{3}H-NA in all regions from 14 to 42 days. However, in the seizure-sensitive strain, there was a marked reduction from 14 to 21 days in the 20 min accumulation of ^{3}H-NA. Recovery of this uptake was observed at 42 days, an age at which the DBA/2J strain has developed resistance to seizures. Kinetic studies are now in progress to analyze the saturation of the 5 and 20 min uptake in both strains. The regional and age-specific alteration in ^{3}H-NA accumulation in the sensitive mice may indicate deficiencies in releasable transmitter which could contribute to the marked seizure sensitivity of the DBA/2J mice at 21 days.

704 INVOLVEMENT OF THE MESOLIMBIC DOPAMINERGIC SYSTEM IN THE DRUG-INDUCED ROTATION OF RATS. P.H. Kelly* and K.E. Moore. Dept. of Pharmacol., Mich. State Univ., E.Lansing, Michigan, 48824.

Adult male rats injected with 8 μg of 6-hydroxydopamine (6OHDA) unilaterally into the caudate nucleus also received bilateral 6OHDA injections (8 μg) into the nucleus accumbens or similar injections of vehicle. In both groups of animals the dopamine (DA) content of the 6OHDA-lesioned striatum was reduced to 35% of that of the unlesioned side. Additionally, the 6OHDA lesion of the nucleus accumbens reduced the DA content of the nucleus accumbens and olfactory tubercle to 25-40% of control. In rats which received only a 6OHDA lesion of one striatum, amphetamine (5 mg/kg, i.p.) and methamphetamine (5 mg/kg, i.p.) produced rotation towards the lesioned side, while apomorphine (0.5-5.0 mg/kg, i.p.) produced rotation in the opposite direction. The bilateral 6OHDA lesion of the nucleus accumbens markedly reduced the amphetamine and methamphetamine-induced rotation but enhanced the rotation produced by apomorphine. These results suggest that drug-induced rotation results from an asymmetry of striatal dopaminergic activity plus activation of mesolimbic dopamine receptors. The *in vivo* effects of dopamine agonists and antagonists in the unilateral 6OHDA lesion model cannot therefore be considered to be solely mediated by effects on nigrostriatal dopaminergic mechanisms. (Supported by USPHS Grant NS 09174).

705 LOCALIZATION OF SEROTONIN NEURONS IN THE RAT HYPOTHALAMUS. Dan Kent* and John R. Sladek, Jr., Dept. Anat., Univ. Rochester Sch. Med., Rochester, NY. 14642. (SPON: B. Tabakoff)

Serotonin (5-HT) has been detected biochemically in the endocrine hypothalamus and is a candidate for involvement in endocrine function. However, the precise compartmentalization of 5-HT remains unclear. Thus, a re-examination of the basal hypothalamus was made. Adult male rats were treated with either saline, a combination of reserpine (R, 10 mg/kg @ 18h), pargyline (P, 100 mg/kg @ 4h), and tryptophan (T, 200 mg/kg @ 2h), or a pretreatment with PCPA methyl ester (200 mg/kg @ 48 & 24h) prior to R, P, and T. Tissues were freeze-dried, treated for formaldehyde-induced fluorescence, and examined in a modified Leitz MPV II microspectrofluorometer. Analysis of arcuate, ventromedial and periventricular nuclei revealed a population of neurons containing yellow histofluorescence. Emission spectra were characteristic of 5-HT (max @ 520, 550 nm). These cells were not visible in saline treated rats, but were demonstrated by treatment with R, P, and T. Pretreatment with PCPA abolished this drug-induced fluorescence. Raphe neurons served as tissue controls. Their fluorescence was dull yellow in saline treated rats, but bright yellow in R, P, and T treated rats with or without PCPA. In all cases, raphe emission spectra were characteristic of 5-HT. PCPA caused a reduction in fluorescence intensity in raphe detectable only by microspectrofluorometry.

The hypothalamic cells may be serotonergic, perhaps linked to the recently described supra/subependymal 5-HT plexus. However, they may be cells capable of accumulating 5-HT, situated postsynaptic to 5-HT terminals. Further studies are in progress to resolve this question.

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706 CATECHOLAMINES AND "AVERSIVE" CIRCLING BEHAVIOR. R.S.Kiser, R.M.Lebovitz, and D.C. German. Depts. of Psychiat. & Physiol. Univ. of Texas Health Sci. Cntr., Dallas, Texas 75235.

Two different types of aversive behavior can be produced by midbrain electrical stimulation in the rat. One type is associated with electrical stimulation of the dorsal midbrain tegmentum (DMT) in the region of the dorsal central gray. This behavior is grossly "fearlike". The other type is associated with stimulation in the ventral midbrain tegmentum (VMT) in the region of the red nucleus. This behavior consists of stereotyped, ipsiversive circling with no obvious fearlike components. Both types of stimulation are aversive in that animals will learn to bar-press to decrement the stimulation current in 5% increments, relative to baseline current. We have previously reported that bar pressing of DMT-stimulated rats is inversely related to brain serotonin levels, whereas the bar pressing of VMT-stimulated rats is unchanged by serotonin manipulations (Soc. for Neurosci.,1974,#353). In this study, 150 mg/kg of the catecholamine-depleting drug alpha-methyl-para-tyrosine (AMPT), or normal saline, were given i.p. to 14 circling (VMT) rats and 14 fearlike (DMT) rats in random order a minimum of one week apart. AMPT strongly reduced the bar pressing of VMT-stimulated rats relative to saline controls, but the drug had no comparable effect in the DMT-stimulated rats. These results suggest that the aversive circling behavior is dependent upon brain catecholamine levels, and that the aversive "fearlike" behavior is relatively unaffected by catecholamine levels.

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707 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF MICRONINJECTIONS OF 6-HYDROXYDOPAMINE INTO THE SUBSTANTIA NIGRA OF THE RHESUS MONKEY. Gary W. Kraemer*, William T. McKinney, George R. Breese, and Arthur J. Prange. University of Wisconsin, Madison, Wisconsin, and University of North Carolina, Chapel Hill, N.C., USA.

Injections of 6-hydroxydopamine bilaterally into the substantia nigra of 10 rhesus monkeys produced whole brain dopamine (DA) depletions of 60-100% of control and norepinephrine (NE) depletions of 40-70% of control. Three behavioral syndromes related to the degree of amine depletion were observed. Four animals (60-70% DA down) were transiently adipsic and anorexic following surgery but recovered in 2-3 days and showed no other effects of the lesion. Four animals (70-85% DA down) showed a caudate turning reflex up to 12 hours after surgery followed by slight rigidity and tremor lasting up to 3 days. These animals were anorexic and adipsic up to 2 weeks following surgery. Two animals (85-100% DA down) showed the caudate turning reflex after surgery for 24-28 hours. This was followed by increasing tremors and rigidity which did not abate over the six months that the animals were observed. These animals showed no recovery from aphagia and adipsia following surgery.

Assay of urinary MHPG revealed no changes in levels of excretion in animals with near total (85-100% DA down; 70% NE down) depletions of central amines.

These preliminary studies establish a model confirming the role of central amine depletions in Parkinsonism. Lack of change in urinary MHPG suggests that this compound cannot serve as an index of brain amine metabolism in primates.

708 THE SOMATODENDRITIC COMPLEX OF NIGRAL DOPAMINE (DA) NEURONS IN ACTIVITY CHANGES. Walter Lichtensteiger, Franz Hefti*, Dominik Felix* and Ruth Lienhart*, Dept. Pharm. and Brain Res. Inst., Univ. Zürich, CH-8006 Zürich, Switzerland.

In urethane-anesthetized rats, a non-linear correlation was observed between mean fluorescence intensity of DA nerve cells as detected by microfluorimetry and mean single unit activity in zona compacta of substantia nigra. Cellular fluorescence intensity varied closely with activity also in time as evidenced in a biphasic reaction elicited by physostigmine. The cellular intensity response to activation appears to depend upon intact amine synthesis as indicated by earlier results with alpha-methyltyrosine. This relationship seems to involve only a fraction of the nigral amine pool, as nicotine did not increase DOPA accumulation after NSD 1015 in the somatodendritic complex. Experiments with cocaine showed that amine uptake is not of major importance for the cellular intensity response to activation. Subcellular redistribution of DA is investigated as an alternative basis of these somatic reactions. Biochemical analysis of substantia nigra requires a differentiation between DA cells and dendrites. The biochemical changes in the latter cannot be inferred from those in terminals. Gammabutyrolactone did not increase DA concentration and reduced synthesis rate checked by DOPA accumulation in substantia nigra in contrast to caudate. This may at least partly reflect the situation in dendrites which contain considerable amounts of DA.

709 CENTRAL CATECHOLAMINES AND GENETIC OBESITY IN MICE. Joan F. Lorden and Gary A. Oltmans*. Dept. Psych., Temple Univ., Philadelphia, PA. 19122.

The intracerebral administration of catecholamines (CA), particularly norepinephrine (NE), produces significant changes in ingestive behavior, both eliciting and suppressing food intake. Lesions of dopamine (DA) neurons also alter feeding and cause prolonged aphagia. This relationship between CA and feeding suggests a possible abnormality in CA function in cases of genetic obesity. To explore this possibility, CA levels in three types of genetically obese mice (the obob, the dbdb, and the viable yellow mutations) were measured. Significant increases in telencephalic and hypothalamic NE levels were found in both the obob (+17 and +34%, respectively) and the dbdb (+21 and +27%, respectively) mice when compared to non-obese littermates. No significant changes in DA were found in either of these mutants. Mice with the viable yellow mutation, however, did not show any significant changes in CA levels in comparison to lean controls. Thus, altered CA levels do not necessarily accompany genetic obesity. However, at the time of sacrifice, the obesity of the viable yellows was not as pronounced as that in either the obob or dbdb mice. Thus, it is possible that altered CA levels in the obob and dbdb mice were a result of stress associated with obesity rather than being involved in producing the obesity. To test this possibility, obob mice were placed on a restricted diet which maintained their body weights at about control levels. This treatment had no effect on CA levels. Telencephalic and hypothalamic NE levels in mice placed on the restricted diet were significantly higher than levels in lean littermates, but did not differ significantly from levels in obob mice with unlimited access to food. Increased levels of amine do not necessarily reflect changes in release, reuptake, or turnover. Analysis of CA turnover did not reveal any significant differences between obob mice and their lean littermates.

710 THE ROLE OF CATECHOLAMINE PATHWAYS IN LITHIUM INDUCED DRINKING. Richard B. Mailman, T. Steven Barlow* and George R. Breese. Dept. of Psychiatry, University of North Carolina School of Medicine, Chapel Hill, N. C. 27514.

The polydipsia induced in rats by daily intraperitoneal injections of lithium chloride was examined in control and catecholamine depleted males. Depletions in norepinephrine, dopamine or both catecholamines were accomplished by appropriate treatment with 6-hydroxydopamine (Pharmacol. Biochem. Behav. 1:319-328 (1973)). Only depleted animals whose water intake was not stimulated by the presence of sucrose in drinking water were selected for the experiments (idem.) Rats received 2 moles lithium/kg body weight and were permitted Purina Lab Chow ad libitum. Control animals consumed increased quantities of water after lithium treatment. Depletion of dopaminergic or both catecholamine pathways countered the effect of lithium, whereas depletion of norepinephrine did not. Parallel experiments with adrenergic agonists/antagonists were consistant with the involvement of dopaminergic pathways in consummatory behavior in rats. The relation of these pathways to renin-angiotensin systems is discussed.

711 NOREPINEPHRINE MODULATION OF PURKINJE CELL RESPONSES TO IONTOPHORETICALLY APPLIED AMINO ACIDS. Hylan C. Moises and Donald J. Woodward. Dept. Cell Bio. Univ. Tx. Health Sci. Ctr., Southwestern Med. Sch., Dallas, Tx.75235.

It is well established that microiontophoretic application of norepinephrine (NE) can inhibit spontaneous discharge of cerebellar Purkinje (P) cells. Recently, it has been proposed that NE may enhance P cell responsiveness to both excitatory and inhibitory afferent input, relative to the level of background spontaneous activity (Hoffer et. al., 1975, Neurosci. Abs., 316). Here we examined whether iontophoresis of NE in low doses acts on the P cell to alter directly its responses to conventional amino acid neurotransmitters. Unit responses of P cells were recorded in Halothane - anesthetized albino rats using multibarrel micropipettes. Effects of microiontophoretic pulses (10-15 sec. duration at 30-40 sec. intervals) of the putative cerebellar neurotransmitters gamma-aminobutyric acid (GABA) and L-glutamic acid (glutamate) were examined before, during and following NE iontophoresis. In 9 of 11 cells inhibitory effects of GABA were potentiated by simultaneous iontophoresis of low doses of NE (5-40 nanoamps), which alone elicited minimal or no depression of spontaneous activity. Augumentation of GABA inhibition often persisted for several minutes after spontaneous discharge had returned to control levels following cessation of NE administration. In 5 of 7 cells a post-excitation inactivation (10-30% inhibition), observed subsequent to termination of threshold glutamate applications (10-40 nanoamps), was selectively enhanced by NE. With cessation of NE administration and recovery of spontaneous discharge, potentiation of both the excitatory and inhibitory response to glutamate microiontophoresis could be found, followed by a gradual return to control levels. These data further suggest that tonic adrenergic input may act to modulate the efficacy of conventional excitatory and inhibitory synapses in the cerebellar cortex. (NSF GB-43301).

712 β-ADRENERGIC RECEPTORS IN RAT CEREBRAL CORTEX: EFFECTS OF 6-HYDROXYDOPAMINE. P.B. Molinoff, J.R. Sporn, *T.K. Harden, B.B. Wolfe,* and B.K. Poulos.* University of Colorado Medical Center, Denver, Colorado 80220.

The intraventricular administration of 6-hydroxydopamine to adult rats (200 ug x 2) leads to the destruction of adrenergic nerve terminals in the cerebral cortex and to an 80% decrease in norepinephrine levels. Similar changes are observed following the subcutaneous administration of 6-hydroxydopamine (100 mg/kg x 4) to newborn rats. The effects of each of these procedures on isoproterenol induced cyclic AMP accumulation and on the density and properties of β-adrenergic receptors have been investigated in rat cerebral cortex. Cyclic AMP formation was measured after labelling tissue ATP pools by incubating brain slices with ^{3}H-adenine [Shimizu et al., J. Neurochem. 16:1609, 1969]. β-adrenergic receptors were measured in homogenates with a high affinity antagonist, (^{125}I)-iodohydroxybenzylpindolol [IHYP; Sporn and Molinoff, J. Cyc. Nuc. Res. 2 (3), 1976]. The accumulation of ^{3}H-cyclic AMP in response to a maximally effective concentration of isoproterenol (30 μM) was 40-80% greater in 6-hydroxydopamine treated rats than in sham treated controls. There was no change, however, in the EC_{50} for isoproterenol stimulation of cyclic AMP accumulation or in the ability of isoproterenol to inhibit the binding of IHYP. The density of IHYP binding sites was determined by Scatchard analysis. There was a 30-50% increase in the density of sites following either subcutaneous administration of 6-hydroxydopamine to newborn rats or its intraventricular administration to adults. These results suggest that the density of β-adrenergic receptors regulates the amount of catecholamine responsive cyclic AMP accumulation and that changes in receptor density are involved in denervation supersensitivity at central noradrenergic synapses. Supported by the USPHS and the American Heart Association.

713 EVIDENCE FOR A DOPAMINERGIC INHIBITION OF NEURONS IN THE PREFRONTAL CORTEX OF THE RAT. F.Mora, K.F.Sweeney*, E.T.Rolls, and A.M.Sanguinetti*, Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, Great Britain.

A mesocortical dopamine system which provides a dopaminergic input to the prefrontal cortex has recently been found in the rat using biochemical, pharmacological and histological techniques. In an electrophysiological investigation of this system, the dopamine-receptor agonist apomorphine (0.016, 0.06 and 0.1 mg/kg) decreased the spontaneous firing rates of neurons in the medial prefrontal cortex, but not of neurons recorded simultaneously in areas thought not to receive a dopaminergic input, the somatosensory cortex and hippocampus. The inhibitory effects of apomorphine lasted for 10-30 min. Similar inhibitory effects were obtained with D-amphetamine (1, and 2 mg/kg) and L-Dopa (100 mg/kg), which increase the release of dopamine from presynaptic terminals. The inhibitory effects found with apomorphine and D-amphetamine were blocked by previous injections of the dopamine-receptor blocking agent spiroperidol (0.1 mg/kg). These findings are consistent with the hypothesis that dopaminergic terminals in the medial prefrontal cortex have an inhibitory function.

714 RADIOAUTOGRAPHIC VISUALIZATION OF CENTRAL MONOAMINE NEURONS AFTER LOCAL INSTILLATION OF TRITIATED SEROTONIN AND NOREPINEPHRINE IN ADULT CAT. A.M. Mouren-Mathieu, L. Léger* and L. Descarries. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec.

Microinjections of 5-HT-^{3}H or NA-^{3}H were carried out in the region of nuclei raphe dorsalis (RD) and locus coeruleus (LC) of adult cats, aiming for a specific radioautographic identification of serotonin (5-HT) and norepinephrine (NA) neurons, respectively. The cats were pretreated with a monoamine oxidase inhibitor, and each tracer was used at a molarity approximating 10^{-3}, alone or in combination with the other non-radioactive amine added at a concentration ten times higher. The amines were diluted in 0.5 µl of saline and instilled through glass micropipettes during 3 h. In light microscope radioautographs of the RD, numerous strongly labeled nerve cell bodies were always observed after injection of either 5-HT-^{3}H alone or in combination with cold NA. Unreactive neurons were interspersed among the reactive cells, and the latter were found in comparable number and distribution from one animal to another. When NA-^{3}H was instilled in RD, a number of cells were labeled in the vicinity of the injection site, but these were no longer visible after concomitant administration of cold 5-HT. In the LC, the injection of either NA-^{3}H alone or NA-^{3}H and cold 5-HT induced an intense reaction of many neurons. However, following 5-HT-^{3}H administration, numerous nerve cell bodies were also found to be weakly or moderately labeled, while some scattered cells were intensely reactive. After injection of 5-HT-^{3}H with cold NA, only heavily labeled scattered cells persisted, suggesting the existence of a few 5-HT nerve cell bodies within LC of cat. Since it seems possible to prevent interspecific labeling, the present method should be applicable for light and electron microscopic examination of 5-HT and NA neurons in any part of brain. (Supported by grant MT-3544 from the MRC of Canada).

715 REQUIREMENTS FOR AND CHARACTERISTICS OF c-AMP STIMULATED TYROSINE HYDROXYLASE. T. Ohashi*, G. S. Drummond*, and M. Goldstein. Dept. of Psychiatry, Neurochemistry Labs, NYU Medical Center, New York, New York 10016.

We have previously reported that % stimulation of tyrosine hydroxylase (TH) by c-AMP was optimal when the enzyme was activated at pH 6.0-6.5 and the tyrosine hydroxylation was carried out at pH 7.1. We have now further investigated the requirements for the stimulation of TH activity by c-AMP. The addition of c-AMP alone does not enhance the striatal or mesolimbic TH activity. NaF or ATP stimulates TH activity in presence as well as in absence of added c-AMP. The addition of exogenous protein kinase results in an enhanced stimulation of TH activity only in presence of added c-AMP. The stimulation of TH activity by NaF might be due to the inhibition of ATPase or of phosphoprotein phosphatase. The % stimulation of TH activity elicited by c-AMP is dependent on the temperature and on the protein concentration of the incubation medium. Histones and other substrates for protein kinase inhibit the c-AMP elicited stimulation of TH activity. The c-AMP activated enzyme is more sensitive to heat denaturation than the native enzyme. The K_m for $DMPH_4$ of the rat adrenal enzyme increases from 0.32 mM to 1.20 mM following partial heat inactivation, and is restored to 0.26 mM by subsequent c-AMP activation. The changes in the kinetic state of the enzyme could imply that the native enzyme is in part present in phosphorylating form and is dephosphorylated following heat inactivation.
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716 HYPERPHAGIA AND OBESITY FOLLOWING NON-SPECIFIC DAMAGE IN THE MIDBRAIN TEGMENTUM OF THE RAT. Gary A. Oltmans* and Joan F. Lorden (SPON: D. L. Margules). Dept. Psych., Temple Univ., Philadelphia, PA. 19122.

On the basis of studies in which the forebrain norepinephrine (NE) of the rat has been depleted by infusions of 6-hydroxydopamine (6-OHDA), it has been suggested that the ascending NE neurons may play a role in the control of feeding and body weight regulation. However, it is now well-demonstrated that intracerebral infusions of 6-OHDA cause non-specific damage in addition to having a specific action on catecholamine containing neurons. Thus, evaluation of the contribution of non-specific damage to the behavioral effects following 6-OHDA lesions is necessary. In the present study bilateral infusions of 4, 8, or 12μg of 6-OHDA·HBr were made in the midbrain path of the ascending NE fibers. Large depletions of hypothalamic (-75%) and telencephalic (-85%) NE were obtained with the 4μg dose. Increasing the dose above 4μg did not cause any significant additional increase in NE depletion. However, increases in non-specific tissue damage were observed. Food intake was significantly elevated over control levels only with the 12μg dose. By increasing the ascorbic acid concentration of the infusion vehicle from .1% to .2%, it was possible to observe increases in food intake with the 8μg dose. Electrolytic, copper sulfate, and 5,6-dihydroxytryptamine lesions at the same site caused increases in food intake and body weight with only moderate (-32 to -58%) decreases in NE levels. Thus, behavioral changes were seen only in those groups which sustained completely non-specific lesions or in which substantial non-specific damage was observed. The behavioral effects were attributed to general tissue damage in the area and not to damage to the NE fibers alone.

717 DRUG-INDUCED ALTERATIONS IN THE FIELD-STIMULATED RELEASE OF ^{3}H-DOPAMINE FROM SLICES OF RAT STRIATUM AND MEDIAN EMINENCE. Nancy A. Perkins* and Thomas C. Westfall. Dept. of Pharmacology, University of Virginia, Charlottesville, VA 22901.

The effect of neuroleptics on the stimulated induced release of ^{3}H-dopamine (^{3}H-DA) from isolated striatal slices is controversial. The present experiments were carried out in an attempt to help clarify the controversy as well as to obtain information on presynaptic regulation of DA release. The effect of various drugs on the electrically-induced release of ^{3}H-DA (biphasic pulses, 2 volts, 5-10Hz) was studied in superfused slices obtained from rat striatum and median eminence. High concentrations ($\sim 10^{-5}$M) of haloperidol, chlorpromazine, promethazine and pimozide increased the electrically-induced release of ^{3}H-DA by 53, 22, 100 and 50%, respectively. On the other hand, lower concentrations ($\sim 10^{-7}$M) produced a significant inhibition of electrically induced release by 22, 32, 25 and 28%, respectively. All these neuroleptics were found to be good inhibitors of ^{3}H-DA uptake with ID50's of 1.25×10^{-6}, 1×10^{-6}, 2.7×10^{-6} and 4×10^{-7}M, respectively, as determined in striatal synaptosomes. Similar results were obtained in experiments utilizing slices from rat median eminence. The dopamine agonist, apomorphine (10^{-5}-10^{-7}M) inhibited the electrically-induced release of ^{3}H-DA from both slices and synaptosomes. Chlorpromazine (5×10^{-7}M) antagonized this effect. Adrenergic agonists, isoproterenol (10^{-5}-10^{-7}), terbutaline (10^{-5}) and clonidine (10^{-5}-10^{-6}) did not alter induced ^{3}H-DA release. It is concluded that neuroleptics have complex, dose-dependent, presynaptic actions on dopaminergic nerve terminals including: a) inhibition of electrically-induced release of DA at low concentrations and b) increased overflow of DA at high doses due to blockade of DA uptake. The present results also support the concept of presynaptic inhibitory DA receptors. (Supported by USPHS Grants #NS10260 and GM12139.)

718 THE CIRCADIAN VARIATION OF BRAIN CYCLIC AMP AND CATECHOLAMINE METABOLISM IN RHESUS PRIMATES. M. Perlow, B. Festoff, M. Ebert, E. K. Gordon, M. G. Ziegler, C. R. Lake, H. Hoffman*, D. K. Johnson* and T. N. Chase, NIH, Bethesda, MD 20014.

Cerebrospinal fluid (CSF) was removed continuously in 2-hour aliquots from the lateral ventricle of the chronic chair restrained rhesus monkey. Under conditions of 12 hours light (0600-1800 hrs) and 12 hours darkness (1800-0600 hrs) the concentrations of norepinephrine (NE), homovanillic acid (HVA) and adenosine 3', 5' monophosphate (cAMP) were found to describe a circadian pattern, with maximal concentrations occurring during the light hours and minimal concentrations occurring during the dark hours. The patterns were generally coincident with the circadian patterns of brain temperature and body activity. When assayed for 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) and 3-methoxy-4-hydroxymandelic acid (VMA), samples of CSF collected over 3 to 4 days demonstrated no reproducible pattern of change. Fluctuations in the concentration of MHPG did not correspond in direction or magnitude to changes in the concentration of VMA. These random fluctuations are most probably established by unknown processes that occur during the time the metabolites diffuse from brain parenchyma to CSF. These results suggest a circadian turnover of dopamine and norepinephrine in the primate brain. A postsynaptic catecholamine responsive adenylate cyclase located in the caudate nucleus is also supported by these in vivo observations.

719 EFFECT OF LERGOTRILE MESYLATE ON DOPAMINE TURNOVER IN RAT BRAIN. Kenneth W. Perry* and Ray W. Fuller. The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206.

Lergotrile mesylate (Lilly 83636) is an experimental drug being evaluated clinically for the treatment of breast cancer, galactorrhea, and Parkinson's disease. Lergotrile is thought to act by direct stimulation of dopamine receptors in the brain and in the pituitary gland, the major effect in the pituitary being suppression of prolactin secretion (J. A. Clemens et al, Acta Endocrinologia 79, 230, 1975). Lergotrile caused stereotyped gnawing behavior in rats similar to that produced by other dopamine agonists such as apomorphine. Decreased dopamine turnover in rat brain after lergotrile injection was indicated by a decline in brain levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and diminution of the fall in dopamine levels following inhibition of its synthesis. Brain DOPAC levels were unchanged at 30-60 min after lergotrile injection (20 mg/kg i.p.) but were decreased at 3-8 hrs. The decrease in DOPAC at 5 hrs occurred at doses of lergotrile as low as 2.5 mg/kg i.p. Lergotrile given at 10 mg/kg 1 hr before α-methyltyrosine methyl ester (250 mg/kg) significantly antagonized the decline in brain dopamine measured at 4 hrs. Whereas apomorphine and piribedil caused rapid decreases in brain DOPAC levels (maximum within 30 min), lergotrile and ergocryptine (another ergoline type of dopamine agonist) produced a slower decline in brain DOPAC (maximum about 5 hrs). The possibility that actions on pre- versus postsynaptic receptors (A. Carlsson, Modern Pharmacol.-Toxicol., Vol 3, p. 49, 1975) are involved in these differences will be discussed. Lergotrile also caused a marked increase in the norepinephrine metabolite MOPEG sulfate and a lesser increase in the serotonin metabolite 5HIAA. These effects were not blocked by spiperone, a dopamine receptor antagonist, which did block the stereotyped behavior induced by lergotrile.

720 DIFFERENTIAL BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF THREE DOPAMINERGIC AGONISTS. R.F. Pfeiffer* Walter Reed Army Medical Center Depart. Neurol Washington, DC, and E.K. Silbergeld, NINCDS, NIH, Bethesda, MD.

Bromocryptine (2-bromo-α-ergocryptine) and lergotrile (d-2-chloro-6-methylergoline-8-acetonitrile methanesulfate) are two ergoline derivatives which have therapeutic action in the treatment of Parkinson's disease. Studies with nigrostriatally lesioned rats indicate that while bromocryptine and lergotrile are dopaminergic agonists, the rotational behavior induced by bromocryptine is partially dependent on intact presynaptic elements (Fuxe, et al Med. Biol. 52, 121, 1974). Bromocryptine and lergotrile were compared to apomorphine to investigate further their actions on dopaminergic pathways. All three induce stereotypy in rats: lergotrile (10 mg/kg) induces as much stereotypy as apomorphine (2-4 mg/kg) and more than bromocryptine (10 mg/kg), based on quantitative scoring of behavior. However, its behavioral effect is of shorter duration than bromocryptine but longer than apomorphine at these doses. Pretreatment with α-methyl-para-tyrosine (200 mg/kg) significantly reduced, by 35%, stereotypy scores of rats given bromocryptine, but did not reduce lergotrile- or apomorphine-induced stereotypy. Uptake and release of ^{3}H-dopamine was studied in rat whole forebrain minces incubated in vitro with the three drugs. Bromocryptine significantly inhibited uptake of dopamine at concentrations 1×10^{-6}M. Lergotrile (10^{-9} to 10^{-8}) increased release 40-50% at a maximum; at higher concentrations, the predominant effect of lergotrile was to inhibit uptake of dopamine. Apomorphine in a range from 10^{-9} to 10^{-5}M did not affect uptake or release in this system. These results suggest that bromocryptine and lergotrile in addition to possessing qualities of dopaminergic agonists may also release dopamine from nerve terminals and inhibit its reuptake.

721 DIFFERENTIAL EFFECTS OF APOMORPHINE ON SELF-STIMULATION OF MESOCORTICAL AND NIGROSTRIATAL SYSTEMS IN RAT AND RHESUS MONKEY. A. G. Phillips, F. Mora*, J.M. Koolhaas* and E.T. Rolls*. Dept Psychology, Univ. British Columbia, Vancouver, B.C. V6T 1WS, and Dept Exptl Psychology, Oxford University, Gt Britain.

Two major dopaminergic systems, the mesocortical system and the nigrostriatal system have been implicated in brain-stimulation reward. As a means of comparing and contrasting the rewarding effects of stimulation in these two systems a series of rats was prepared with electrodes in the terminal regions of both systems, i.e., medial and sulcal prefrontal cortex, and neostriatum. The dopaminergic agonist apomorphine (0.075, 0.15, 0.3, 0.6 and 1.2 mg/kg) produced a significant dose-related inhibition of self-stimulation in both medial and sulcal cortex. In contrast, a facilitation of caudate self-stimulation was observed at neostriatal sites in the majority of animals. Further confirmation of the differential effects of apomorphine was obtained in rhesus monkeys with self-stimulation electrodes in orbitofrontal cortex and caudate nucleus. Self-stimulation in the orbitofrontal cortex was inhibited by apomorphine (0.1, 0.2, 0.4 mg/kg) whereas a marked facilitation of caudate self-stimulation was observed at the two lower doses in the same animals on the same test day. These data suggest the direct involvement of a dopaminergic mechanism in self-stimulation of prefrontal cortex and a modulatory role for dopamine in brain-stimulation reward in the neostriatum.

722 PROJECTIONS FROM RAPHE NUCLEI TO CEREBELLAR CORTEX IN CATS. E. Taber Pierce, F. Walberg, and G.H. Hoddevik*. Dept. Anatomy, Harvard Med. Sch., Boston, Mass. 02115 and Anatomical Institute, Oslo, Norway.

The efferent connections of the raphe nuclei to the cerebellar cortex were determined for 75 cats following injections of 0.005-0.5 μl of horseradish peroxidase (Sigma VI, 50% wt/vol in buffered saline) into selected areas of the cerebellar cortex. The brains were perfused with 0.4% paraformaldehyde and 1.25% gluteraldehyde with 0.05M phosphate buffer (pH 7.4), immediately dissected out, cut into blocks, reimmersed into more of the same fixative, rotated at 4°C overnight, transferred to a phosphate buffer with 30% sucrose and rotated 24 hours at 4°C. Frozen blocks were cut at 50-60 μ, collected in groups of 5 in distilled H_2O and treated according to the HRP method of Graham and Karnovsky (1966). Cresyl violet and unstained sections were studied with light and dark field and interference contrast. Neurons in raphe nuclei obscurus, pallidus, magnus, pontis, and dorsalis project in variable numbers to specific cerebellar cortical sites. Certain nuclei project more to specific folia than others. Some folia apparently receive no afferents from the raphe nuclei. Nuclei raphe obscurus and raphe pontis are the main source of afferents to the cerebellar cortex. In all cases studied most HRP positive cells in the raphe appear less reactive than the bulk of the cells in other nuclei known to project to the cerebellar cortex (inferior olive, griseum pontis).

723 EFFECTS OF p-CHLOROPHENYLALANINE AND METERGOLINE ON A DELAYED RESPONSE TASK AND SEROTONIN SYNTHESIS WITHIN THE CAT BRAIN. Andrée G.-Roberge and Claire Boisvert*. Laboratoires de Neurobiologie, Département de Biochimie Faculté de Médecine, Université Laval, Québec, Qué., Canada G1K 7P4.

To determine whether or not serotoninergic systems might affect the performance of a delayed response task, p-chlorophenylalanine (250 mg/kg, i.p.) and metergoline (14 µg/kg i.m.) were injected to cats as a single dose and as a chronic treatment. Delayed response performance (the number of errors) was not significantly disturbed by p-chlorophenylalanine and metergoline. However, a significant increase in the time-limit (time in seconds taken by the cat to answer) was observed in cats treated with p-chlorophenylalanine but not in cats treated with metergoline. The same dissociation was observed after L-DOPA and L-5HTP administration whereas the performance of a delayed response task was not changed, but according to the doses used, the time-limit was slightly or significantly increased. The concentrations of serotonin and tryptophan hydroxylase activity were measured in several structures belonging to the limbic system and to the motor system at different periods of time following drug administration. Metergoline, in spite of decreasing the serotonin content to the same extent as p-chlorophenylalanine within the brain, seems to involve different neurochemical mechanisms. Results are discussed in relation to the biochemical equilibrium between the neurotransmitters within the CNS and to short term memory mechanisms.

(Supported by a grant from MRC of Canada).

724 CENTRAL CATECHOLAMINERGIC EFFECTS ON BRAIN GLUCOSE CONSUMPTION. William J. Schwartz & Frank R. Sharp. Lab. Neurophysiol., NIMH, Bethesda, MD 20014

We have studied the changes of brain glucose consumption which result from lesions of CNS dopamine (DA) pathways (6-hydroxydopamine injections in substantia nigra) and noradrenaline (NA) pathways (6-hydroxydopamine injections in locus coeruleus). Brain glucose consumption was determined using the [^{14}C] 2-deoxy-D-glucose tracer method, a method devised and used quantitatively by Sokoloff (Science 187: 850).

Unilateral 6-hydroxydopamine (6-OHDA) lesions aimed at the substantia nigra in rats result in decreased glucose consumption of structures rostral and ipsilateral to the lesion as compared with that of the contralateral side. Regions affected include frontal cerebral cortex, caudatoputamen, and other subcortical structures. Controls show no such asymmetries.

Unilateral 6-OHDA lesions of the locus coeruleus in rats result in a change from control in the relative rates of glucose consumption in the layers of the cerebellar hemispheres. In lesioned animals, the glucose consumption of the cerebellar granular layer is greater than that of the molecular layer. This pattern is not present in controls, in which the glucose consumption of cerebellar granular and molecular layers in the hemispheres appears equal. In addition to this change, the glucose consumption globally throughout the lesioned brains appears increased bilaterally compared with that of controls. This overall increase of glucose consumption is larger in cerebellum than in forebrain.

Even though DA and NA are both generally believed to be inhibitory neurotransmitters, our results suggest that their ultimate actions on brain metabolism are opposite.

725 LESIONS OF THE LOCUS COERULEUS AND MATING BEHAVIOR IN MALE RATS.
G. Rufus Sessions*, J. C. Salwitz*, and G. J. Kant (SPON: R. M. Wylie), Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D. C. 20012.

Rostral midbrain lesions that interrupt the dorsal noradrenergic bundle (DNB) and deplete cortical or telencephalic norepinephrine (NE) by 63% have recently been reported to dramatically reduce the refractory post-ejaculatory interval (PEI) in male rats (Clark et al., Science, 1975, 190, 169). It was concluded that this increase in sexual vigor could have resulted from the destruction of the DNB or from destruction of nearby anatomical structures unrelated to the DNB. The present study examined the copulatory behavior of rats in which the DNB was discretely lesioned at its source in the nucleus locus coeruleus (LC) in the dorsal pons. Male Wistar-derived rats were given three copulatory tests before receiving bilateral electrolytic lesions of the LC (N = 5) or sham-lesion operations (N = 4). Standard measures of copulatory behavior were recorded for two copulatory series on each test: mount and intromission latencies; ejaculation latency; mount and intromission frequencies; mean interintromission interval; and postejaculatory interval. After a two-week recovery period testing was resumed and three postoperative tests were conducted, separated by one week each. Cortical NE was reduced by 51-91% (mean = 65%) in the lesioned animals, but no lesion-related changes in mating behaviors were observed. PEI measures were normal as compared with the Shams. These results suggest that increased sexual vigor resulting from rostral midbrain lesions may be unrelated to the interruption of the DNB and depletion of telencephalic NE.

726 PRODUCTION OF CONTRALATERAL ROTATION BY INJECTION OF DOPAMINERGIC AGENTS AND CATECHOLAMINES INTO THE DOPAMINE-DEPLETED CAUDATE.
Paulette E. Setler, Katherine L. Turner* and Maryann R. Malesky.* Dept. Biol. Res., Smith Kline & French Labs., Phila., PA 19101.

Rats with a 6-hydroxydopamine-induced lesion in one substantia nigra rotate in the direction contralateral to the lesion following systemic administration of dopaminergic drugs such as L-dopa or apomorphine (APO). To test whether the rotation is due to direct activation of supersensitive denervated dopamine receptors, a cannula was implanted in the denervated caudate for intra-caudate (i.c.) injection of drugs. Drugs were injected slowly in 2 µl and the rats immediately placed in hemispherical cages in which rotations were automatically counted for 2 hours by means of a microswitch attached to the rat by a lightweight harness. Catecholamines and serotonin were given with a monoamine oxidase inhibitor. Dose-related rotation was produced by dopamine (DA) and norepinephrine, which were approximately equipotent producing maximal rates of rotation at about 1.0 µg, and also by epinine, epinephrine, APO and 2-amino-6,7-dihydroxytetralin. Serotonin caused moderate rotation which was not dose-related. Isoproterenol, amphetamine and clonidine were ineffective. Piribedil and its metabolite S584 were weakly active at high doses.

The results support the suggestion that rotatation is due to direct activation of denervated dopamine receptors. The degree of agonist supersensitivity caused by denervation is indicated by the fact that in the lesioned rat the effect of 0.5 µg of DA is equivalent to the effect of 7.5 µg of DA given into the caudate of the non-lesioned rat. It is not possible to determine from these data to what extent the loss of catecholamine uptake sites contributes to the supersensitivity observed, nor to what extent supersensitivity in this preparation is a manifestation of postsynaptic change following denervation.

727 ASCENDING CATECHOLAMINERGIC PATHWAYS AND LATERAL HYPOTHALAMIC SYNDROME IN THE RAT. M. S. Shahid Salles* and W. E. Gladfelter. Dept. of Physiol. & Biophysics, West Virginia University Med. Ctr., Morgantown, WV 26506.

To investigate whether the lateral hypothalamic syndrome results from the destruction of ascending catecholaminergic pathways, bilaterally symmetrical electrolytic and chemical (6-hydroxydopamine) lesions were made at several sites along the course of these pathways (substantia nigra, ventral tegmental area, and lateral hypothalamus) and their effects on spontaneous locomotor activity, food and water intakes, and body weight were studied. In these experiments, rats on an ad libitum feeding-drinking regimen were housed individually in cages with activity wheels located in a room kept at a temperature of 21 $\pm$ 2°C. For each rat, records were kept of its spontaneous locomotor activity, food and water intakes, and body weight for 8 weeks before and after the placement of bilateral lesions at one of the forementioned sites. Only rats with electrolytic lesions in the lateral hypothalamus had decreases in food and water intakes and in spontaneous locomotor activity that lasted for the duration of the experiment. Rats in which the ascending catecholaminergic pathways were destroyed caudal to the hypothalamus and rats in which the pathways were destroyed in the lateral hypothalamus by 6-hydroxydopamine (a chemical which is reported to specifically destroy catecholaminergic pathways in the amounts we used) had, at most, only temporary decreases in food and water consumption and in locomotor activity. Thus, these data do not support the hypothesis that the lateral hypothalamic syndrome results from interrupting the ascending catecholaminergic pathways. This research was supported by a WVU School of Medicine GRS Grant # 5 S01 RR05433-14.

728 A PUNCH SAMPLING TECHNIQUE FOR QUANTITATIVE MEASUREMENT OF TRITIATED d-AMPHETAMINE IN DISCRETE AREAS OF MONKEY BRAIN. Donna M. Simmons*, Peter H. Blake*, and Douglas M. Bowden. Regional Primate Research Center, University of Washington, Seattle, Washington 98195.

In recent experiments, d-amphetamine was injected intraventricularly in awake monkeys to test its effects on intracranial self-stimulation behavior. In order to identify possible sites of drug action, tritiated d-amphetamine was used in the terminal experiment, and its distribution in specific structures was determined by a punch sampling technique.

Autoradiographic methods used to visualize other tritiated compounds in whole brain sections are not applicable to amphetamine because of its high water solubility. Radiochemical assay techniques can be used to identify tritiated amphetamine, but the procedures are complex and time consuming, and require homogenization of large amounts of tissue. For these reasons, a technique of tissue sampling, solubilization, and scintillation counting was developed to identify tritiated amphetamine in discrete areas of monkey brain. The method is rapid and simple. The brain is divided into 1-cm coronal slabs which are cut in 1 mm sections. Multiple punch samples (1.5 mm^3) are taken from individual periventricular structures to determine concentration gradients of amphetamine.

The procedure consists of six steps: obtaining tissue blocks, preparation for sectioning, punching and sectioning, histological verification of sampling sites, and analysis of scintillation count data. Using this procedure, a steep gradient of radioactivity was demonstrated in caudate nucleus, septum, and other periventricular structures.

729 PERISTENCE OF NIGROSTRIATE TRANSMISSION AND DOPAMINE-INDUCED INHIBITION OF CAUDATE NUCLEUS NEURONS IN THE PRESENCE OF EXTRAPYRAMIDAL DYSFUNCTION CAUSED BY HALOPERIDOL IN CATS. G.G. Somjen, P. Zarzecki, D. Blake*. Dept. of Physiology and Pharmacology, Duke Univ., Durham, N.C. 27710

It is generally believed that the extrapyramidal motor system side effects of butyrophenones are caused by blockade of dopaminergic synaptic transmission in the nigrostriate pathway. We tested this theory in 12 cats treated with haloperidol until their voluntary movements were severely disturbed (10 to 20 mg/kg/day for 5 to 30 days). The symptoms were hesitancy and slowness of movement, abnormal postures and a fine rest tremor of the head and trunk. Nigrostriate synaptic transmission was studied by recording single units and evoked potentials and by microiontophoresis in the caudate nucleus of unanesthetized cats. In animals with movement disorders, stimulation of the substantia nigra by stereotactically placed electrodes inhibited 52%, excited 24% and did not influence 24% of caudate nucleus neurons. The proportion of cells influenced by nigral stimulation was not statistically different from those found in 40 untreated control animals. Dopamine released from microiontophoretic electrodes inhibited 71% of the cells found in the caudate nucleus of treated cats. This proportion, and the currents required to achieve an effect, were not different from those found in control animals. Neither the amplitude nor the waveshape of the nigrostriate evoked potential were altered by the administration of a single dose of haloperidol (6 to 20 mg/kg i.p.). The evoked potentials were not significantly different in chronically treated animals. We conclude that the motor disturbances induced by haloperidol were not caused by blockade of dopaminergic nigrostriate synaptic transmission.

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730 AMPHETAMINE POTENTIATION OF LOCOMOTOR ACTIVITY AFTER KNIFECUT LESIONS OF ASCENDING CATECHOLAMINE-CONTAINING NEURONS. C.A. Sorenson and D.B. Kee*, Psychology Department, Amherst College, Amherst, MA 01002

The locomotor stimulating effect of amphetamine is generally believed to be due to action of this compound on central catecholamine (CA)-containing neurons, particularly ascending dopamine (DA) and norepinephrine (NE)-containing neurons. However, studies using thalamic (Huston and Borbély, Physiol. Behav., 1974, 12, 433-448) and decerebrate (Baez and Petroff, Neuro. Abs., 1975, 5, 244) preparations have reported that amphetamine produces behavioral activation in these animals. These results seem to suggest that descending NE-containing neurons are involved in the activating effect of amphetamine, but they must be interpreted cautiously, since it is difficult to say what components of the potentiated activity of a reduced preparation represent locomotor behavior. Therefore, the effects of amphetamine on running wheel activity were investigated in rats whose ascending CA pathways were eliminated by bilateral knife cuts in the midbrain. This preparation locomotes normally, and after a few days of recovery resumes normal feeding behavior. We found a) that lesioned animals showed increased locomotor activity to all doses (1.0-4.0 mg/kg) of d- or l- amphetamine administered, b) that there were no differences between the d- and l- isomers of amphetamine in the lesioned group, while d- was approximately three times as potent as l- in the control group, and c) that pimozide, a DA-receptor blocker, abolished the activating effect of 1.0 mg/kg d-amphetamine in control animals but failed to do so in the lesioned group. These results strongly support the hypothesis that descending NE neurons play a role in the locomotor activity produced by amphetamine. They cast considerable doubt on the hypothesis that nigrostriatal DA-containing neurons are primarily responsible for this response to amphetamine.

731 ENTRY OF ^{3}H-NOREPINEPHRINE AND ^{125}I-ALBUMIN FROM BLOOD INTO BRAIN FOLLOWING OSMOTIC OPENING OF BLOOD-BRAIN-BARRIER IN THE RAT. C.L. Sun*, C.C. Chiueh*, I.J. Kopin, W.R. Fredericks*, and S.I. Rapoport (SPON: P. Cardon). NIMH, Bethesda, MD. 20014.

Osmotic opening of blood-brain-barrier (BBB) was effected by infusion of L(+) arabinose (1.58 M in saline, 37°C, 7.4 ml/min for 30 sec) into right internal carotid artery of pentobarbital anesthetized rats. Osmotic shrinkage of cardiovascular endothelium causes reversible opening of tight intercellular junctions and increases transfer of albumin-bound Evans blue into brain (Rapoport et al., Amer. J. Physiol. 223:323, 1972). Therefore, Evans blue (0.5 ml, 2%, i.v.) was utilized as a visual tracer for the opening of BBB. After arabinose infusion, Evans blue was visualized in caudate nucleus, cerebral cortex, hippocampus, thalamus, hypothalamus and midbrain ipsilateral to the site of infusion. A narrow blue region was also found in the contralateral cingulate cortex. No blue color was noted in the brain stem. Immediately after arabinose infusion, ^{125}I-albumin (10 µc) was injected i.v. or ^{3}H-norepinephrine (^{3}H-NE, 25 µc in 2 ml saline) infused over 5 min. Animals were sacrificed 60 min after ^{125}I-albumin and 10 min after ^{3}H-NE. The content of ^{125}I or ^{3}H in the perfused brain was increased by 3-9 fold and 2-4 fold, respectively. The brain-blood ratio of ^{125}I was 0.2 and the perfused brain contained 0.67% of the injected dose/gram. Perfused brain contained only 0.09%/gram of the injected tritium while the heart retained 2%/gram. These results suggested that although the hypertonic solution facilitated entry thru the BBB of ^{3}H-NE as well as ^{125}I-albumin or Evans blue, but the entry of ^{3}H-NE into brain remains small.

732 BRAIN STIMULATION REWARD ASSOCIATED WITH STIMULATION OF THE SUPRACALLOSAL BUNDLE. M. Takigawa*, A. Robertson* and G. J. Mogenson, Departments of Physiology and Psychology, University of Western Ontario, London, Canada.

The supracallosal bundle, the rostral extension of the dorsal noradrenergic pathway through which axons of the locus coeruleus project to the cerebral cortex and hippocampus, was stimulated in acute and chronic experiments to investigate the role of this pathway in brain stimulation reward. In rats anaesthetized with urethane, 32 neurons, histologically verified as being in the locus coeruleus, were antidromically activated by stimulation of the supracallosal bundle. The latency of the antidromic spikes was 45.3 ± 2.0 msec and the conduction velocity of the axons of the locus coeruleus was estimated to be 0.40 - 0.55 msec. When electrodes were implanted chronically in the same area of the supracallosal bundle from which locus coeruleus neurons had been activated, self-stimulation was observed for 14 of 20 electrodes. The rates of self-stimulation varied considerably depending on the location of the electrodes in the supracallosal bundle. Caudal sites, as compared to rostral sites, were associated with slower rates of self-stimulation and elicited motor responses. Electrophysiological recordings were made at the time of sacrifice. Stimulation of supracallosal bundle sites, which had been demonstrated as positive for brain stimulation reward, antidromically activated neurons in the locus coeruleus (latency, 42.3 ± 3.7 msec). The results are consistent with the hypothesis that the noradrenergic axons of the locus coeruleus are associated with brain stimulation reward. (Supported by the MRC and the NRC of Canada)

733 LEAD BLOCKADE OF NORADRENERGIC INHIBITION IN CEREBELLAR PURKINJE NEURONS D. Taylor*, J. Nathanson*, B. Hoffer*, L. Olson*, and A. Seiger*, (SPON: D. Puro). NIMH, Saint Elizabeths Hospital, Washington, D. C. and Karolinska Institute, Stockholm, Sweden.

Biochemical studies showing that lead (Pb^{++}) inhibits cerebellar adenylate cyclase (IC_{50}=2μM; Nature 255, 419, 1975) prompted us to test the effects of this cation on the depression of spontaneous discharge of Purkinje (P) cells produced by iontophoresis of norepinephrine (NE). Previous studies have suggested that the effects of NE on P cell discharge may be mediated by activation of a NE-sensitive adenylate cyclase. Iontophoresis of Pb^{++} in situ, and in cerebellar transplants in oculo, reliably antagonized NE responses in over 80% of the P cells studied in both preparations. Blockade was readily seen at iontophoretic currents of 5-10 nA. Superfusion of Pb^{++} (10 μM) into the anterior eye chamber also antagonized NE responses in P neurons of the transplant. The NE responses showed recovery within 20 minutes after cessation of Pb^{++} administration. Spontaneous discharge rate was either unaffected or slightly elevated at Pb^{++} levels that almost completely blocked NE. Barium, the heaviest metal we studied that does not inhibit adenylate cyclase in vitro, did not block NE effects. Stimulation of parallel fibers or iontophoresis of acetylcholine (ACh) excited P cells. No antagonism was seen, however, between Pb^{++} and these ACh or parallel fiber excitations. Basket cell inhibition, believed to be mediated by GABA, was similarly not reduced. These results raise the possibility that specific blockade of brain catecholamine receptors may partially underlie the CNS toxicity seen with lead administration. Supported in part by Swedish MRC (04X-03185).

734 DEXAMETHASONE INHIBITION OF ETHANOL EFFECT ON BRAIN BIOGENIC AMINES. Charles A. Walker and Karam F. A. Soliman*. Florida A and M University, School of Pharmacy, Tallahassee, FL 32307.

Norepinephrine (NE) dopamine (DA) serotonin (5-hydroxytryptamine, 5HT) levels were measured in different regions of the brain in relation to ethanol (ETOH) and dexamethasone (DEX) administration. In this experiment male Sprague Dawley rats were maintained under controlled light (12L:12D) and temperature (23 ±1). ETOH was administered (2 g/kg) 1 hour following DEX (2 mg/kg) injection. The animals were sacrificed at 1 hour intervals for 4 hours. The cerebral hemisphere, caudate nucleus, midbrain, cerebellum and pons were separated and analyzed for brain biogenic amines content. ETOH administration resulted in a significant ($P \leq .01$) increase in all biogenic amines analyzed in different parts of the brain studied except for 5HT and 5HIAA in the cerebellum and pons. DEX administered along with ETOH was found to block rises in the biogenic amines resulting from ETOH treatment. Meanwhile, DXM administration alone resulted in significant increases ($P \leq .01$) in dopamine concentration in the cerebellum and pons at the 3rd and 4th hours respectively. Most changes in biogenic amines in DXM treated group were noticed after the 2nd hour. The study shows that DEX protection against ETOH toxicity might be mediated through dexamethasone effects on brain biogenic amines. (Supported by a Grant from NASA).

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736 ANATOMICAL AND ELECTROPHYSIOLOGICAL EVIDENCE FOR A PROJECTION FROM THE HABENULA TO THE MIDBRAIN RAPHE IN THE RAT. Rex Y. Wang and George K. Aghajanian. Depts. Psychiat. and Pharmacol. Yale Univ. School of Med., New Haven, CT 06508

By a retrograde tracing method in which horseradish peroxidase (HRP) was iontophoretically ejected into the midbrain raphe nuclei, we found of all brain areas which send afferents to the midbrain raphe the lateral habenula (Hb) contained the highest density of HRP-reactive neurons. A direct Hb-raphe pathway was further demonstrated by the fact that antidromic action potentials could be evoked in Hb cells (latency: 10-30 msec) by stimulation of the dorsal raphe nucleus (DRN). To investigate the physiological properties of the Hb-raphe pathway, extracellular recordings were made from cells in the DRN during Hb stimulation and iontophoresis of suspected transmitters and their antagonists. Electrical stimulation of the Hb markedly inhibited spontaneous firing of identified serotonergic (5-HT) and other neurons in the DRN (latency: 10-30 msec) but had no effect on cells in the midbrain reticular formation. When the stimulating electrode was outside the Hb (2mm lateral to midline) the inhibitory responses of DRN cells could not be obtained. The inhibitory effect of Hb stimulation was totally eliminated by bilateral lesions of the fasciculus retroflexus and adjacent areas, the path taken by DRN-Hb projections. Inhibition of DRN cells produced by either Hb stimulation or iontophoresis of GABA was blocked by I.V. injections of the GABA antagonist picrotoxin (4-6 mg/kg), but not by the glycine antagonist strychnine (0.4-0.7 mg/kg). We conclude that the Hb has a marked inhibitory influence upon neurons in the DRN and that the inhibitory effect might be mediated by a direct Hb-raphe GABAergic pathway. These results support the concept that the lateral Hb is a major nodal point in funneling information from forebrain limbic areas to the midbrain raphe. (USPHS grant MH-17871 and the State of Connecticut).

737 A POSSIBLE ROLE OF THE DORSAL PERIVENTRICULAR BUNDLE IN ANALGESIA: A HISTOCHEMICAL AND BEHAVIORAL STUDY. S. Watson, H. Akil*, and J. Barchas. Dept. Psychiatry, Stanford Univ., Stanford, CA. 94305

Recent modifications of the catecholamine histofluorescence technique has allowed the description of a dorsal periventricular bundle (DPB) running from the periaqueductal grey through the thalamus via the periventricular nucleus (Lindvall & Björklund, *J. Comp. Neurol.*, 1974). However, the function of this system has not yet been clearly delineated. While many investigators have reported analgesia in central grey and medial thalamus with morphine microinjections (Pert & Yaksh, *Brain Res.*, 1974) and electrical stimulation (c.f. Liebeskind *et al.*, *Adv. Neurol.*, 1974) the specific relation to DPB was never specifically examined.

Sixteen rats were each implanted with 2 bipolar electrodes, one along DPB and the other in a control site 2 mm laterally. Changes in pain responsiveness (tail flick) upon electrical stimulation were systematically studied for each site. Bipolar square pulses (200 μsec, 20 Hz in 200 msec trains, 3/sec) were administered at increasing amplitudes until changes in pain responsiveness were observed or 3 ma of current was reached. Stimulation of all medial sites produced reliable analgesia of varying potency. Only two of the lateral sites produced minor analgesia. Histofluorescence by glyoxylic acid (Watson & Barchas, *Psychopharm. Comm.*, 1975) was then performed on the brains. Using blind ratings, there emerged a strong positive correlation (r=.89) between the potency of analgesia and electrode proximity to DPB. Some of the lateral electrodes appeared to recruit non-DPB catecholamine pathways, but did not produce analgesia. It is suggested that the periventricular bundle may be specifically involved in pain regulation. It appears reasonable to expect that the newly discovered morphine-like peptides, enkephalins (Hughes *et al.*, *Nature*, 1975) may closely interact with this system.

738 DIFFERENTIAL INVOLVEMENT OF THE NIGROSTRIATAL AND MESOLIMBIC DOPAMINE SYSTEMS IN SPONTANEOUS AND AMPHETAMINE INDUCED ACTIVITY. David Wirtshafter*, Karen E. Asin and Ernest W. Kent. (SPON: J. D. Davis) Dept. Psychol., Univ. Il. at Chicago Circle, Chicago, Il. 60680.

Anatomical data suggest that the majority of forebrain dopamine is localized within either the mesolimbic or the nigrostriatal systems. Lesion and pharmacological studies have implicated dopamine in a variety of behavioral adjustments but little is known as to the identity of the dopamine systems mediating these various behaviors. To clarify this matter we selectively destroyed portions of the mesolimbic or nigrostriatal systems by injecting 6-hydroxydopamine (40μg in 8μl of ascorbic acid vehicle) bilaterally into either the caudate nucleus (CD) or the olfactory tubercle (OT).

Transient aphagia and adipsia followed CD injections only. Consistent with the hypothesis that the extrapyramidal side effects of neuroleptic agents are due to a blockade of striatal dopamine receptors, injections of 6-hydroxydopamine into the CD, but not the OT, were found to produce catalepsy and occasionally tremor. Activity was measured in a photocell cage 4, 8, and 12 days following surgery. Although CD injections were found to produce a more severe reduction in spontaneous activity than did OT injections, only the latter were effective in blocking amphetamine induced hyperactivity. CD but not OT placements were capable of attenuating the stereotypy induced by 10 mg/kg d-amphetamine. These results suggest that the mechanisms involved in amphetamine induced activity may not be identical to those involved in spontaneous activity.

739 APOMORPHINE INCREASES GLUCOSE UTILIZATION IN THE SUBSTANTIA NIGRA AND SUBTHALAMIC NUCLEUS OF RATS. Leslie I. Wolfson* and Lucy Brown*
(SPON: Gerald Golden). Dept. Neurology, Albert Einstein Coll. Med., Bronx, N.Y. 10461.

In experiments with the dopamine (DA) agonist apomorphine (APO), glucose utilization was used as an index of neural activity (Kennedy and co-workers, Science, 187:850, 1975). Since DA release inhibits neuronal activity in the caudate, it was felt that APO treatment would result in decreased glucose utilization in this region. Contrary to expected results, the major changes noted were in the substantia nigra (SN) and the subthalamic nucleus.

Apomorphine was administered I.V. (4 mg/kg), followed 1 min later by an I.V. pulse of ^{14}C-deoxy-D-glucose to 2 restrained and 2 freely-moving awake rats. After 30 min, rats were decapitated and the brains quickly frozen. Coronal sections of whole brain were cut on a cryostat and then incubated against x-ray film for autoradiographic analysis.

Compared to control rats, the SN in all 4 APO rats showed a striking increase in optical density (isotope concentration) relative to adjacent structures. The only brain area with a similar increase in density was the subthalamic nucleus. When examined under the fluorescence microscope, the subthalamic nucleus showed clusters of catecholamine varicosities. Other DA rich structures such as the caudate nucleus did not exhibit detectable density changes.

These results may be explained either as a direct effect on DA receptor sites or as an indirect effect reflecting the major activity changes within a complex set of neural interactions. In either case, the role of the subthalamic nucleus in the nigrostriatal system and DA-mediated behavioral effects merits further consideration. (Supported by NIH Grants NS 09649 and MH 06418.)

740 CYTOCHEMICAL ANALYSIS OF STRONGLY INTENSELY FLUORESCENT (SIF) CELLS OF THE PELVIC VISCERA GANGLION. Joe Wood, Dept. Neurobiol. and Anat. The Univ. of Texas Med. Sch. at Houston, Houston, TX 77025. William G. Dail, Dept. of Anat. Univ. Mew Mexcio, Albuquerque, NM 87131,

The SIF cells of autonomic ganglia have heretofore been studied by fluorescence microscopy for biogenic amine localization and attempts have been made to correlate the fluorescent cells with those containing dense core granules by conventional electron microscopic methods. The SIF cells of pelvic visceral ganglion contain norepinephrine (NE). This has been determined cytofluorometrically. In the present study,SIF cells were identified by fluorescence microscopy while correlative tissue were processed for electron microscopic localization of biogenic amines. Granules within the SIF cells have the appearance of catecholamine storing granules in the NE cells of the adrenal medulla. To determine whether or not these granules contain NE, thin(500A) sections were examined using the analytical electron microscope where a 100A electron beam was directed toward the reactive sites and the subsequent X-Ray emissions were studied on a multichannel analyzer. The granules are highly positive for chromium and when compared with in vitro preparations of NE plus glutaraldehyde and dichromate, the X-Ray emissions are similar. The small beam available with scanning transmission electron microscopy made it possible to discriminate reaction product from granule to granule and in some cases, partially filled granules were analyzed on an intragranular basis. Areas within cells immediately adjacent to granules are nonreactive and thus, osmiophylic areas which are non- NE containing are differentiated from NE structures. This methodology provides a correlate of a specific light microscopic method with another specific ultrastructural method and definitely identifies amine organelles. It also provides a method whereby minute foci of biogenic amines can be evaluated at a relatively quantitative level. (Supported by NSF-10326).

741 EFFECTS OF L-DOPA ON SEROTONIN-CONTAINING NEURONS OF MOUSE BRAIN. S.M. Wuerthele, C.C. Chiueh*, G. Zeldes and K.E. Moore. Dept. of Pharmacol., Mich. State Univ., East Lansing, Michigan, 48824.

L-dopa decreases the concentration of serotonin (5HT) and increases the concentration of 5-hydroxyindole acetic acid in mouse brain (Everett, G.M. and Borcherding, J.W., Sci. 168:849, 1970), suggesting effects on 5HT synthesis and storage. Mice were injected s.c. with the peripheral aromatic L-amino acid decarboxylase inhibitor Carbidopa (hydrazino-methyl-dopa; 50 mg/kg). Thirty minutes later D- or L-dopa (100 mg/kg) was administered s.c., and mice were sacrificed at various times thereafter. A pulse label of ^{3}H-tryptophan (0.5 μCi/g) or saline was administered i.v. 15 minutes prior to sacrifice. Whole brains were assayed for dopa, 5HT and dopamine, and for ^{3}H-tryptophan and ^{3}H-5HT. Results show that: (1) L-dopa causes temporally related increases in dopamine, and decreases in 5HT concentrations; (2) L-dopa but not D-dopa, causes a dose-related decrease in 5HT concentration; (3) L-dopa has no effect on ^{3}H-tryptophan concentration; (4) L-dopa decreases synthesis of 5HT, which follows a time course similar to the increase in L-dopa concentration in brain. It is concluded that L-dopa causes an initial inhibition of 5HT synthesis which is temporally related to the accumulation of dopa in the brain. In addition, L-dopa causes a longer-lasting decrease in 5HT concentration, which is temporally related to an increase in brain dopamine concentration. (Supported by USPHS Grant MH 13174.)

742 HUMAN PLASMA CONCENTRATION OF PHENYLETHYLAMINE, PHENYLETHANOLAMINE AND TYRAMINE. R. J. Wyatt, J. Moyer-Schwing,* and F. Karoum, Laboratory of Clinical Psychopharmacology, SMR, IRP, NIMH, St. Eliz. Hosp., Wash., D.C. 20032.

The reduced platelet monoamine oxidase activity in some schizophrenics and the possible genetic implication of this observation (Science, 179: 916, 1973) has prompted us to develop a highly sensitive and specific mass fragmentographic method for the simultaneous assay of phenylethylamine (PEA), phenylethanolamine (PEOA) and tyramine (TY) in plasma. The amines were extracted from 0.5 ml plasma into ethyl acetate at pH 12. (To obtain this pH, plasma was mixed with 1 ml of a phosphate buffer prepared by mixing 3 parts 0.3 M Na_3PO_4 with 1 part 0.3 M Na_2HPO_4). Aliquots of the ethyl acetate were evaporated *in vacuo*, reconstituted in 0.3 ml 1N HCl, and mixed with 5 ml ethyl acetate; the ethyl acetate discarded and 0.2 ml of the aqueous phase transferred into a microflex tube and evaporated under nitrogen. The amines were derivatized by heating at 70°C for 20 mins with 15 μl of 10% pentafluoropropionyl imidazole in ethyl acetate. Separation was achieved on a 12-ft column packed with 0.5% OV_{22} + 1% OV_{210} + 2% SE 54 on 80/100 mesh chromosorb W(HP) support (Pierce Chemical, Rockford, Ill.) The extraction procedure and column employed are critical for the accuracy and reproductivity of the assay. Deuterated isotopomers of the three amines were used as internal standards. The concentrations (mean ± SEM) in 12 subjects were 0.59 ± 0.17 ng PEA/ml; 2.8 ± 0.33 ng PEOA/ml, and 4.36 ± 1.6 ng TY/ml of plasma. The concentrations of these three amines range from 0.15 to 2.5 for PEA, 1.4 to 5.3 for PEOA and 2 to 18 for TY (ng/ml).

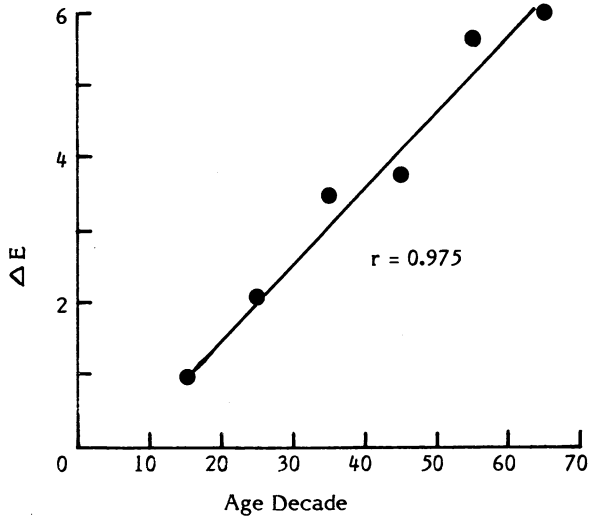
Motor Systems

743 POWER SPECTRAL ANALYSIS OF THE HUMAN POSTURAL CONTROL SYSTEM F. Owen Black, Conrad Wall, III and Dennis P. O'Leary Dept. of Otolaryngology, Eye and Ear Hospital, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213.

Postural control movements of normal subjects have been studied using a Kistler model 9261A quartz crystal force platform connected to a PDP-11 computer system. The subject stands on the platform performing tasks such as the tandem Romberg (TR) maneuver. (In this maneuver the subject stands heel-to-toe with the dominant foot behind. The hands are clasped, the elbows are extended to the side and the subject pulls his arms in tension.) The computer calculates the projection of the subject's center-of-mass onto the horizontal plane of the platform and plots the displacement of this point from the mean value as a vector $\vec{r}$. The magnitude, r, is taken as a measure of the stability of the postural control system. In order to maintain stable posture a subject must keep r below a critical value. Variations in r may be considered a measure of the work the subject is doing to remain stable. Fluctuations in r were measured over an ensemble of normal subjects grouped according to age decade by computing power spectra of each time series r(t), and then ensemble averaging these spectra for all subjects in the same age group. This technique has the advantages that a confidence interval can be assigned to the ensemble power spectrum for normal versus abnormal comparison. The ensemble power spectrum can also be integrated as a measure of the average energy used by a subject in a particular decade age group. We have defined this integral as the average energy estimate $\hat{E}$. The estimate, $\hat{E}$, was computed for six age groups under two test situations: TR with the eyes open and TR with the eyes closed. The change, ΔE, from the two test conditions is defined as

$$\Delta E = \frac{\hat{E}_{closed} - \hat{E}_{open}}{\hat{E}_{open}}$$

and is shown in Figure 1. This quantity increased with increasing age and was fitted by linear regression with a correlation coefficient of 0.975. We interpret this finding as suggesting that the extra energy, ΔE, required to maintain upright position is a linear function of age. (Supported in part by a grant from The Deafness Research Foundation.)



744 CEREBELLAR PATHWAY FOR PRECENTRAL RESPONSES FOLLOWING ARM PERTURBATIONS.
V. B. Brooks, J. Hore, J. Meyer-Lohmann*, T. Vilis. Dept. of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

Many precentral neurons of the monkey respond to arm perturbation with an early discharge (peak 20-50 msec) whose intensity reflects sensory input and whose timing is such that it could contribute to the "functional stretch reflex" (M2 EMG response) (1,2,3). A second precentral response (peak 50-100 msec) has also been recorded that was found to be correlated to the direction of the intended movement rather than the direction of the imposed perturbation (2). Later precentral unit discharges were found to be related to movement oscillations (1,3).

Does the cerebellum provide pathways for (a) the early precentral response that is related to the perturbation and (b) the second precentral response that may reflect central programming? Previous studies with dentate cooling have ruled out neocerebellar participation in the early response, but did not exclude a pathway through n. interpositus (IP).

We now report similar experiments with cooling of IP made with 2 Cebus monkeys that had been trained to resist unexpected perturbations of the arm while holding a handle in a learned target zone, or while moving it from one target zone to the other. The magnitude of the early response in 47/58 precentral units remained unchanged during IP cooling, making it very unlikely that spinocerebellar transmission is important for generation of the precentral early response. In contrast, the second response (peak 50-100 msec) was decreased by IP cooling in 25/44 units. IP-cooling was unfortunately not restricted to that nucleus, but also affected the medial aspect of the dentate. Therefore we cannot distinguish between a path for the second precentral response ascending by a spinocerebellar route, or one that depends on relay of the early precentral response through the cerebellum back to motor cortex.

In view of the correlation of second responses with "set" (2), our present results may reflect a role of the cerebellum in central programming of movements.

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745 THE ORDER OF RECRUITMENT OF HUMAN MOTOR UNITS USING NERVE STIMULATION. W. F. Brown, H.A. Kadrie* and H.S. Milner-Brown*. Dept. of Clinical Neurol. Sciences, University Hospital, London, Ontario, Canada N6A 5A5.

In healthy humans, isometric contraction recruits motor units (MU) in an orderly manner from small to large units at progressively higher force contraction levels. The reverse to the above, using electrical stimulation might be expected in view of the generally accepted evidence that large fibres have lower thresholds to direct stimulation than the smaller myelinated fibers.

In this investigation, 2 methods, isometric contraction and multiple point stimulation have been used to isolate and measure the parameters of MU's in healthy humans. The latter method used graded electrical stimulation at 10-20 distinct points along the length of the nerve to activate the first 1-4 successive motor units at each point in the order of the critical all or nothing firing levels of the units; care being taken to detect and exclude situations in which the firing levels of 1 or more motor units overlapped. Unexpected was the observation that the first MU's excited by near threshold electrical stimulation were not large but small MU's. Large motor units that could be recruited at a high force levels of contraction were not usually activated by threshold stimulation. In keeping with the progressively larger p-p surface voltage of motor units activated at higher stimulus intensities was the observation that the MU's had successively shorter latencies; the results being highly significant using rank correlation and t tests of significance.

The observations established, at least among the first 2-5 motor axons activated by percutaneous stimulation, that motor units are excited in order from small to large though to this date the factors that govern that order of excitability have not been investigated.

746 THE EFFECT OF CROSS-INNERVATION OF ANTAGONISTIC FORELIMB MUSCLES ON EMG PATTERNS DURING LOCOMOTION IN THE RAT. Avis H. Cohen. Section of Neurobiol. and Beh., Cornell University, Ithaca, N.Y. 14853.

In weanling rats the nerves to antagonistic muscles--medial head triceps and long head biceps--were reciprocally deflected. The medial head nerve in these animals was highly complicated, containing what appeared to be many sensory fibers. No attempt was made to separate the components at the time of surgery. In this preparation the result of nerve deflection was varying degrees of dual-innervation to both whole muscles. Experimental animals were examined 3-6 months after nerve cross. The function of the doubly innervated medial head triceps was assessed during unrestrained locomotion by simultaneous EMG recordings from it and the adjacent normal long head triceps which served as the control muscle. In normal rats these two forelimb extensors have been shown during unrestrained locomotion to fire essentially at the same time, while the forelimb flexors fire after the extensors have ceased activity (Cohen and Gans, J. Morph., 146: 177-196, 1975).

The dual-innervated medial head triceps showed one of two characteristic EMG patterns. 1) The more common pattern contained both flexor and extensor bursts of activity. That is, in a step cycle there occurred activity both synchronous with and out of phase with the long head triceps activity. However, the period between extensor bursts of both control and experimental muscles of the limb was considerably longer than normal. 2) The less common EMG pattern contained only extensor bursts, even though the dual-innervation to the muscles was later confirmed. The period between extensor bursts was within normal limits in these animals.

In all crossed animals tested electrophysiologically, activity was evoked in the dual-innervated muscles in response to electrical stimulation to either the original or foreign nerves. Thus, in this preparation both sets of neuromuscular junctions appear to be functional.

The behavior of the long head biceps and the gross movement of the limb are still under study.

747 ACTIVITY PATTERNS IN FAST AND SLOW MUSCLES DURING SELECTED FORELIMB MOVEMENTS. T.C. Collatos*, V.R. Edgerton, and J.L. Smith. Dept. Kinesiology and Brain Res. Inst., UCLA, Los Angeles, Ca. 90024

Recruitment patterns of fast and slow synergists in the cat forelimb were studied. The elbow extensors examined were characterized, one as fast and the other slow, on the basis of their histochemical profiles and contraction times. These muscles, the superficial lateral triceps (LTT) and the accessory portion of the medial triceps (MTA), are both fusiform and uniarticular extensors.

Reduced nicotinamide adenine dinucleotide diaphorase and acid labile myosin adenosine triphosphatse activities were used to determine the fiber type populations of both muscles. The LTT is composed of 85% fast-twitch fibers, 63% fast-twitch-glycolytic (FG) and 22% fast-twtich-oxidative-glycolytic (FOG), while the MTA consists of 80% slow-twitch fibers leaving 14% FOG and 6% FG. Whole muscle contraction times taken from 5 animals corroborate the fast and slow nature of these 2 muscles as indicated by their myosin ATPase activities. Mean times-to-peak-tension for the LTT and MTA were 34 ± 2.4 and 74 ± 21.5 msec respectively.

To determine the recruitment patterns of the fast LTT and slow MTA during normal forelimb movements, electromyograms from muscles of 2 cats were recorded using chronically implanted electrodes. Myopotentials were transmitted by a telemetry unit which permitted the cat complete freedom of movement. The cat's movements and the corresponding EMG signals were synchronized by a special effects generator and recorded on video tape for display on a single monitor.

During quiet quadrupedal standing with the elbow joint moderately flexed, both slow and fast muscles were recruited synchronously, although fewer units in the fast muscle were involved. As the cat trotted at 1.25 m/sec on a treadmill, motor units in both muscles were activated simultaneously and minimally prior to paw touchdown. Both muscles remained minimally active during the yielding phase of the stance (E_2), but during the propulsive phase (E_3), EMG activity in the slow MTA diminished to baseline while electrical activity of the fast LTT persisted and peaked (see Fig. 1). The durations of the activities in fast and slow muscles were 170 and 90 msec respectively.

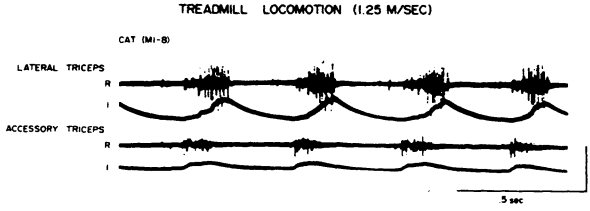

Figure 1: Integrated and raw EMG for 4 step cycles.

When one of the forelimbs was lifted as the cat swiped at an object suspended above her head, activity in the MTA was minimal while a burst of activity was observed in the LTT.

We conclude that single joint synergists of quite different biochemical and contractile properties are selectively activated. The pattern of activation is characteristic of the type of movement. The small, slow contracting extensor, MTA, participates only during postural type activities. Conversely, the larger, fast contracting LTT shows bursts of activity during propulsive and rapid movements.

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748 TWO NEURAL PROCESSES CONTROLLING RESPONSES OF HUMAN SUBJECTS TO LOAD CHANGE
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Normal human subjects show powerful load compensating responses which occur at intermediate latencies, ones longer than the monosynaptic reflex but shorter than the usual reaction time. These responses have been attributed to supraspinal servo-loops. It has further been shown that the intermediate-latency response is subject to volitional modification, as tested with different instructions. The modifications have been attributed to a change in the gain of the supraspinal servo-loop. Our experiments were designed to test an alternative interpretation of these data, the notion that the intermediate-latency, modifiable responses might represent "voluntary" reactions, even though the latencies are shorter than those associated with usually cutaneous and visual reaction tasks.

Mechanical disturbances in force were delivered to the human arm at random times; arm acceleration and displacement, and biceps EMG, were monitored as response. Each subject was studied in four situations consisting of combinations of (1) the instruction "compensate" or "do not intervene" and (2) disturbances of random or of known direction. Within the early post-stimulus interval, there were no differences in response related to instruction; differences developed abruptly after a latency which ranged from 70-320 ms (acceleration traces), in different subjects. This result with disturbances of constant direction was in agreement with previous studies; e.g., the modifiable responses of elbow flexors studied by Hammond had 70 ms latencies in force traces. We also studied the latency of modification when the direction of the disturbance was made random. In this situation, the direction of a correct response was unknown to the subject prior to the onset of the disturbance, and this difference in protocol caused the latency of the modifiable response to become longer (10-50 ms) and more variable. We also noted errors in response direction, which appeared to represent incorrect choices by some decision-making process in the brain.

These findings suggest that the intermediate-latency, modifiable response is a triggered choice reaction analogous to a voluntary reaction, and not a servo action. When triggered reactions were suppressed (usually successful with the instruction "do not intervene"), the responses which remained were simple deflections of the arm in the direction of the disturbance. These compliant responses were regulated by a servo action which we attribute to the stretch reflex. We concluded that a rigid regulation of muscle length is not a fundamental reflex function; instead, it appears to represent a more sophisticated control strategy that may or may not be superimposed upon a compliant reflex response by the production of prompt triggered reactions. The latter are preprogrammed movements rather than compensatory servo actions.

749 MOTOR CORTEX DISCHARGE ASSOCIATED WITH PRECISELY CONTROLLED FINE MOVEMENTS. C. Fromm* and E. V. Evarts. Lab. Neurophysiology, NIMH, Bethesda, MD. 20014.

"Craik's Ratio Rule" states that over a wide range of movement magnitudes the size of movement error is proportional to the size of the movement. How does the motor cortex participate in "scaling" precisely controlled movements? This is the question that was posed in the present experiment.

Monkeys were trained to make precisely controlled arm movements with a visual-tracking paradigm in which they viewed two horizontal rows of lamps (a track row and a target row). Each row contained 11 lamps, with one and only one lamp in each row being illuminated at any one time. Each lamp in the track row was directly beneath a corresponding lamp in the target row. The lamp which was on in the "track" row corresponded to the orientation of a handle which the monkey grasped and positioned by pronation-supination arm movements. A $1\frac{1}{2}^{o}$ handle movement shifted the track display from one lamp to the next. Thus, a movement of 15^{o} moved the track display from one lateral extreme to the other. The monkey was rewarded for maintaining alignment between target and track lamps, and when there was a misalignment the monkey would make a pronation-supination movement whose direction and magnitude corresponded to the direction and magnitude of the misalignment. Misalignments resulted either from target shifts (under control of the experimenter) or from track shifts occurring with a fixed target when the monkey was seeking to maintain alignment but failed to do so. Contralateral arm area units were recorded in the standard manner, but no pyramidal tract electrode was placed and so units were not identified as pyramidal tract neurons.

Precentral arm area units whose discharge was related to performance of this task fell into two broad categories: 1) Tonic Units discharged while alignment was being steadily maintained but tended to become less active during correction of misalignments. These units ceased to discharge tonically, and sometimes became totally silent during rapid, ballistic rotations of the handle, as occasionally happened when the monkey became satiated and was no longer motivated to perform the task properly. 2) Phasic Units showed bursts of activity in relation to the dynamic components of misalignment corrections. These units usually showed increased discharge for one direction of movement but not for the other. Typically, they showed bursts prior to and during movement, with about the same response latency for movements triggered by the target jumps or by the track shifts occurring when the target zone was overshot or when steady alignment was broken by handle "drift." Comparisons of unit discharge for large (12^{o}) and small ($1\frac{1}{2}^{o}$) movements triggered by large or small target jumps showed more prolonged discharge during the longer-lasting (and higher velocity) large movement. The velocity records of the large movements were not those of "ballistic" movements, but instead contained a number of peaks, and striking correlations were seen between these peaks and successive peaks in the instantaneous frequency of unit discharge. Peak discharge frequencies were not significantly greater for the 12^{o} than for the $1\frac{1}{2}^{o}$ movements. These findings will be discussed in relation to the hypothesis that the phasic units constitute elements of a "movement pattern generator" which uses its full dynamic frequency range regardless of the intended magnitude of a movement. Such a notion implies that there must be a mechanism for scaling (up or down) the relation between output of the pattern generator and the muscle response, which is, of course, greater for the longer, higher velocity movements.

750 SIZES, INTRACORTICAL LOCATIONS AND PROPERTIES OF MAJOR NEURONAL OUTPUT POPULATIONS IN THE ARM AREA OF PRIMATE MOTOR CORTEX. D. R. Humphrey, W. S. Corrie* and R. Rietz*, Lab. Neurophysiol., Emory Univ. Sch. Med., Atlanta, GA 30322.

To obtain data that will be useful in planning future functional studies of the primate motor cortex, microelectrode recording techniques were used to examine the properties of pyramidal tract (PT), corticorubral and corticoreticular neuron populations within the precentral gyrus of the anesthetized rhesus monkey. The cells were identified by antidromic activation, and the investigation was confined to a small cortical zone, some 2 mm in surface diameter, whose major motor effects are exerted upon the flexor and extensor muscles of the contralateral wrist. Each cell system was studied with regard to intracortical location, neuronal packing density, distribution of axonal conduction velocities and major axonal destination. Recordings were obtained from over 1,600 antidromically identified cells, and the data were corrected numerically for sampling errors. Our major results may be summarized as follows.

(i) On a numerical basis, the major outflow from the cortical arm area appears to come not from its widely studied population of large PT cells, but instead from: (a) a much larger population of small, slowly conducting PTNs, 75 % of which send their axons to the contralateral cord; and (b) a separate population of small corticorubral cells, which exert excitatory synaptic effects upon rubrospinal tract neurons. Together, these two populations appear to account for close to 90 % of the cells within this area of the cortex that might be expected to participate in any reasonably direct way in the control of wrist movements.

(ii) The majority of the cells within each projection system were concentrated within cortical layer V. No tendency was found for a separate spatial clustering of fast and slow neurons, as has been claimed for PT cells within the forelimb area of the cat's motor cortex.

(iii) When the estimated packing densities for all cell populations were combined, however, they appeared to account for less than 40 % of the cells within layer V. Thus, layer V appears to contain many cells that are either interneurons, or efferent cells that project to structures that are not directly involved in the production of wrist movements.

Though basically simple anatomical observations, these findings appear to be of considerable significance for the design of future studies of the cortical control of movement and posture. Many of our current concepts of the function and somatotopic organization of the motor cortex appear, for example, to be based heavily upon observations of the activity and synaptic actions of its small subset of large PT cells. Moreover, in a number of previous studies of the behavior of cortical cells in conscious animals, it has been assumed that the majority of the unidentified cells that are isolated in the deeper layers of the motor cortex are in fact efferent neurons, that may participate rather directly in the control of limb movements. In view of our present findings, neither of these assumptions (or practices) appears to be prudent. Instead, our results emphasize the importance of anatomically identifying neurons in future single unit studies of motor cortex behavior, and of focusing upon the activity of the small neurons, which, though difficult to record from, are numerically dominant.

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751 LATENCY OF VOLUNTARY BREATHING REACTIONS TO AIRWAY OCCLUSION IN MAN.
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External obstruction of the airway at the beginning of inspiration results in isometric contraction of the inspiratory muscles and a rapid change in intrapulmonary pressure measured at the mouth. The early pressure change (< 150 msec.) has been proposed by Whitelaw et al. (Respir. Physiol. 23: 181, 1975) as a useful index of respiratory center output and sensitivity since it is easy to measure and is unaffected by pulmonary mechanics or by volume-related vagal reflexes. I have conducted experiments with 9 normal young adults to find out how soon after occlusion the pressure wave can be affected by voluntary reactions to the load or by voluntary breathing in anticipation of the load.

When subjects attended to their breathing and tried to inspire as quickly as possible after sensing that the airway was occluded, reaction times (RTs) ranged from 160 to 480 msec. (group average 294 msec.) The faster RTs were associated with increased negative pressure developed in the first 100 msec of the obstructed breath. Selected subjects were trained to make voluntary inspirations of varying flow rates against resistive loads. When occlusions occurred at the onset of a maneuver which produced a rapid pressure change, RTs were shorter than for voluntary efforts resulting in slow pressure changes. These RT changes can best be ascribed to changes in respiratory stimuli since attentional level was reasonably constant and finger responses to occlusion showed comparable RT shifts. For optimal conditions of practice, attention and respiratory stimuli, 150 msec. was the shortest occlusion RT for inspiratory muscles; this confirms the conclusion of Whitelaw et al. that the occlusion pressures occurring at 100 msec. are free from the intrusion of voluntary reactions to the loading stimuli.

When a subject's attention is successfully distracted from rhythmic automatic breathing by listening to a radio, performing a visual vigilance task, or drowsing, the occlusion pressure wave has a characteristic negatively accelerated form with little variability from trial to trial in the first 150-200 msec. The entire course of the pressure wave, however, becomes highly variable with marked alterations in waveform, 1) during the first presentations of occlusion stimuli, 2) when instructed to pay attention to breathing, 3) under distraction conditions when subjects report they are unable to breathe in the apparatus without attending to their breathing. In some subjects the change in occlusion pressure waves are predictable from the intervening breathing pattern which has come under voluntary control. Under these attentional conditions the voluntary reactions are also variable and "false" RTs of 0 to 100 msec. may be recorded if a reaction is begun in anticipation of the load.

I have observed no short latency pressure transients that might represent "load compensating reflexes" (50-70 msec) of inspiratory muscles when voluntary breaths were occluded. This is not surprising in view of the large fraction of inspiratory pressure contributed by the diaphragm which has not been shown to be capable of this kind of rapid adjustment during voluntary breathing tasks

Our data suggests that the occlusion method for estimating respiratory center output is limited more by attentional conditions which can lead to voluntary breathing and load anticipation than by rapid voluntary reactions to the obstruction.

752 LOAD COMPENSATION THROUGH CNS CONTROL OF PROPRIOCEPTIVE INTERACTION WITH CENTRALLY GENERATED MOTOR ACTIVITY. John D. Marrelli*, and William H. Evoy. Dept. of Quantitative Biology, Univ. of Miami, Coral Gables, Florida, 33124.

An understanding of the control of posture and movement can be obtained through the study of the reflex responses to the perturbation of voluntary movements. Changes seen in the reflex response dependent upon the behavior should give some insight into the CNS- Peripheral Nervous System interaction in the production of normal behavior. The Merus-Carpus (MC) joint of the crayfish, Procambarus clarkii, was forced to flex and extend continuously through 45° at a frequency of .38 Hz. by a high stiffness dc motor. The influence of the movement about the joint on the discharge of the motoneurons to the muscles controlling that joint angle was studied for three different central nervous system states; steady-state posture, attempted flexion and attempted extension. Mechanical stimulation of groups of sensory hairs or electrical stimulation of single interneurons (command fibers) produced either flexion or extension about the MC joint. During one cycle of the imposed movement the flexor and extensor muscles are alternately stretched. The averaged frequency of reflex discharge of the extensor (Y_E) and flexor (Y_F), motoneurons was found to be directly proportional to the VELOCITY of the imposed movement, ($\dot{X}$), plus some background level of discharge (B). This relationship may be expressed by: $Y_E = M_E.X + B_E$ and $Y_F = M_F.X + B_F$ for the extensor and flexor motoneurons where B is intercept value at 0 velocity (background discharge) and M is the slope of the line and the gain of the reflex response to limb perturbation with units of (DISCHARGE RATE/UNIT CHANGE IN VELOCITY).

As might be expected, during the active movements of the MC joint, B_E and B_F change reciprocally. That is, when the animal initiates a flexion movement the background discharge in the flexor (B_F) rises and that in the extensor (B_E) falls. This can be interpreted as simple summation between the central drive to the motoneurons and the proprioceptive feedback from the regular movements about the MC joint.

However, the coefficients M_E and M_F also change reciprocally. When active extension is produced the value of M_E rises to twice its value for no movement, while the value of M_F falls almost to zero. Active flexion produces reciprocal changes in these coefficients. M_F and B_F rise and M_E and B_E fall. These changes occur whether the movements are produced through sensory hair or command fiber stimulation.

Changes in the slope (gain) of the reflex response dependent upon the direction of centrally initiated movement indicates that the CNS not only drives the motoneurons but also reciprocally modulates the sensory feedback to the motoneurons dependent upon the direction of movement. The behavioral significance of these changes seems clear. If no change in reflex gain occurred upon active flexion, the stretch of the extensor muscle by the movement would produce proprioceptive responses tending to drive extensor and inhibit flexor motoneurons. This tendency is reduced by a reduction in M_E. However, if the flexor muscle is stretched during its active contraction, the response of the flexor motoneurons is greatly enhanced as M_F is greater at this time. Active movement in the extension direction is similarly dealt with. Thus, it would seem that the capability of effective load compensation is maintained and even enhanced during active movements while proprioceptive information tending to retard the active movement is specifically suppressed through the interaction of the CNS with the sensory to motor pathway. The control of the crayfish cheliped MC joint is therefore seen to be a form of adaptive control in which the characteristics of the control system change to suit the behavior.

753 BIOMECHANICAL AND CINEMATOGRAPHICAL ANALYSIS OF A SERIAL MOTOR ACT. EFFECTS OF DORSAL COLUMN SECTION. M. McCormack* and B. Dubrovsky. Neurophysiol. Lab., Allan Mem. Inst., McGill Univ., Montreal, Quebec.

In previous work we have studied the effects of dorsal column section in a serially organized motor act (Exp. Brain Res., 18, 165, 1973), requiring cats to jump up in order to release a piece of liver attached to a vertically oriented revolving wheel. Although the behavioral studies showed significant impairment in the efficiency, accuracy and in tracking after dorsal column section, the method employed could not provide direct information concerning the mechanisms underlying the deficits. Studies of the mechanical effort of the animal complemented by film analysis of the flight phase of the jump before and after surgery might provide information concerning these processes. Animals were trained to jump from a force platform equipped with three transducers which registered the three vectorial forces (vertical and both horizontal components) involved in the jump. Each component of the act was then measured.

After dorsal column section the following parameters, all directly related to the force, were significantly decreased: 1: height of jump, 2: time in the air, 3: maximum resolved force , and 4: peak to mean force ratio (an indication of the speed at which the cat developed the maximum muscular force). The timing of the jump was evaluated by measuring the take-off time of the cat from the platform in relation to the position of the wheel. After surgery the animals started the jump with a significant delay and greater variability.

Film analysis showed that intact cats consistently extend their limbs in a smooth and progressive way towards the liver in order to release it. The distance between the tips of the forelimbs remained more or less constant during the flight. Postoperatively, instead of the regular pattern described, the extension of the limbs was interrupted by fast flexion movements and the distance between their tips increased significantly during the flight.

Impairment in force development after surgery may relate, in part, with section of fibres from skin mechanoreceptors conveying information on loading conditions on the limbs. Marsden et al (Nature, 238: 140, 1972) have shown the important role of cutaneous afferents for load compensation in proportion to force and suggested a transcortical mechanism for its development. Further , we think that dorsal column section supresses an important path for cutaneous afferents involved in supraspinal mechanisms of synchronization of muscle activity (Milner Brown et al, Electroenceph. clin. Neurophysiol., 38: 245, 1975). These authors suggested that the mechanisms for exertion of large, brief forces (e.g. jumping) may result from strengthening reflex pathways involving a fast, lemniscal route to the cortex. The impairment described in reaching the target postsurgically may be associated with the fact that the dorsal columns are the exclusive afferent path to brain centers for muscle spindles and for low threshold joint afferents from the forelimbs. Also, decreased cutaneous information coursing through the columns should hamper accuracy in forelimb projection as skin sensations are essential for refined motor acts, (Granit, R., Brain, 98, 550, 1975). Before surgery the animals jumped up with a consistent stereotype pattern including the direction of take-off. After dorsal column section cats not only started the jump with delay and more variation in relation to preoperative timing, but also there was a significant change in the direction in which the cat took off, suggesting a change in the strategy of the cat while on the ground.

754 RECURRENT INHIBITION DURING LOCOMOTION. David A. McCrea* and Larry M. Jordan. Dept. of Physiol., Univ. of Manitoba, Winnipeg, Manitoba R3E OW3.

Although it is well established that the spinal cord is capable of generating locomotor activity, the neuronal nature of the generator remains to be elucidated. Neurones which are rhythmically active during locomotion in the absence of afferent input include motorneurones, Ia inhibitory interneurones (Feldman and Orlovsky, Brain Res. 84:181, 1975), and ventral spinocerebellar tract neurones (Arshavsky et al, Brain Res. 43:276, 1972). All of these cells are subject to inhibition through the Renshaw cell pathway (Renshaw, J. Neurophysiol. 9:191, 1946; Hultborn et al, J. Physiol. 215: 613, 1971; Lindstrom and Schomburg, Acta Physiol. Scand, 88: 505, 1973), suggesting that Renshaw cells can influence the output of the spinal stepping generator. Severin et al (Bull. Exp. Biol. Med. 66:5, 1968) suggested that depression of Renshaw cells could be responsible for their finding that the effectiveness of antidromic inhibition of ventral root discharges was reduced during locomotion. Previous work from this laboratory has shown that the excitability of Renshaw cells is not decreased during locomotion. This report describes the effect of locomotion on recurrent inhibitory postsynaptic potentials (R-IPSP) recorded in motorneurones. Locomotion in thalamic cats occurred spontaneously or in response to a brief stimulus to a cut dorsal root (Budakova, Neurosci. Behav. Psych. 5:355, 1971) and was monitored by recording rhythmic discharges in ventral root filaments which occurred in phase with locomotion. Rhythmic filament discharge persisted following paralyzation with Flaxedil ("fictive" locomotion; Perret, Thesis, CNRS A 08342, Paris, 1973) and occurred either spontaneously or following a stimulus such as swinging of a forelimb. It was found that antidromic stimulation of the L_7 ventral root could induce R-IPSP's in motorneurones during fictive locomotion, and the size of the R-IPSP varied as the membrane potential of the motorneurone varied, being larger in the depolarizing phase than in the hyperpolarizing phase. This evidence, together with our previous finding that Renshaw cell excitability is not decreased during locomotion, suggests that Renshaw cells can still exert their inhibitory actions upon motorneurones during locomotion.

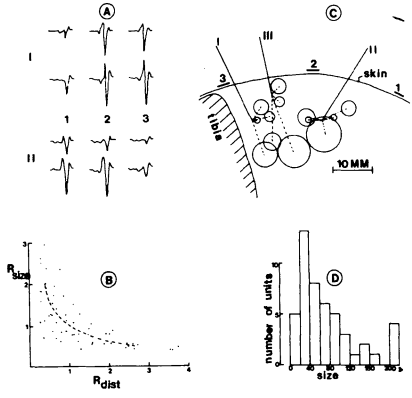
In order to determine whether Renshaw cell activity is essential for locomotion, we administered mecamylamine, a drug capable of blocking all but the first few Renshaw cell spikes evoked by a single antidromic ventral root stimulus (Ueki, et al, Exptl. Neurol. 3:141, 1961). Decerebrate cats were induced to walk on a treadmill by stimulation of the midbrain locomotor region (Shik et al, Biofizika 11:879, 1966). Mecamylamine (4.0 mg/Kg i.v.) never abolished evoked locomotor activity, although in some experiments it transiently raised the threshold for evoking locomotion. Thus, the bursting type of Renshaw cell activity observed in response to an antidromic ventral root stimulus is not essential for locomotion. We cannot rule out the possibility that the few Renshaw cell spikes remaining after mecamylamine are physiologically important and may in fact be essential for locomotion. (Supported by M.R.C., Canada)

755 SPATIAL LOCATION AND SIZE DISTRIBUTION OF MOTOR UNITS IN LARGE HUMAN MUSCLE. W. Monster and H. Chan. Temple Univ. Sch. Med., Phila., PA. 19140.

The surface electromyogram potentials of human tibialis anterior motor units were recorded at multiple locations along a crossection of the muscle. Units were discharged volitionally and under near-isometric conditions. Discharging units were detected with a thin intramuscular needle electrode (Disa Type 13K0592). The potential sizes of each unit at the multiple surface electrodes were extracted from the total surface electromyogram using evoked-response averaging. The intramuscular potential of a unit served as the trigger in the averaging process. Fig. A shows the potentials of 4 units at three surface electrodes (1, 2, 3). These units were isolated at two needle positions (I and II). At each needle tip location typically 4 to 7 units of different size and threshold could be differentiated by simple peak detection. Using on-line data processing it was possible to sample 60 or more units from 10 to 15 needle locations at 8 surface electrode positions in each of 10 experiments.

The sizes (and, to some extent, the shapes) of the unit surface potentials within one crossection reflect the average distance of each unit's muscle fibers relative to the surface electrodes. In the expectation that units recorded at a given needle tip location, on the average, would tend to be centered in the vicinity of that location, units were grouped accordingly. For each group of units, the average ratio (R_{size}) of the potential sizes at two surface electrodes was plotted against the ratio (R_{dist}) of the distances from these surface electrodes to the needle tip location. Fig. B shows that the potential sizes within a group decay monotonically with increasing distance to a surface electrode. It also follows that a unit's muscle fibers are, as expected, not distributed uniformly throughout the muscle; units recorded at a given needle location, on the average, tend to be grouped around that location. This pattern of localized spread was clearest for the numerous (Fig. D) small units; variability of the data points in Fig. B was further reduced by using needle tip locations somewhat distant (e.g.>5mm) to the muscle's crossectional boundaries.

The relationship in B (dashed line) suggested a means to estimate, for each unit, the average geometric center of its fibers within the muscle crossection. This estimate was based on the units surface potential sizes at three or more electrodes placed around the circumference. The result for three groups of estimates is shown in Fig. C. Unit sizes in Fig. C are normalized to a fixed distance from the center surface electrode (circle diameter = potential size). Units of different size were found to be fairly uniformly distributed throughout the muscle. The normalized sizes also were used to obtain the size distribution of the sample (Fig. D).



756 MONOSYNAPTIC EXCITATION AND INHIBITION OF NECK MOTONEURONS BY A RETICULOSPINAL PATHWAY. B.W. Peterson, N.G. Pitts*, R.G. Mackel* and K. Fukushima*. The Rockefeller University, New York, N.Y. 10021.

Motor actions of reticulospinal pathways have been investigated in detail only in hindlimb motoneurons (1,2,3). Since other descending systems establish different patterns of connections with neck and limb motoneurons (4), we chose to study reticulospinal actions on neck motoneurons.

Excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) were recorded intracellularly from motoneurons supplying neck extensor (splenius, biventer cervicis, complexus) and flexor (occipitoscapularis) muscles in cerebellectomized cats anesthetized with chloralose. Motoneurons were identified by antidromic activation and were then studied to determine their responses to 100-150 μA, 100 μs pulses applied to the brain stem through bipolar concentric electrodes located 2-3 mm below the floor of the fourth ventricle. Arrays of 8-12 electrodes were used to stimulate points ranging from 0-11 mm anterior to the obex and from 0-4 mm to each side of the midline. When necessary, depolarizing current was injected through the recording electrode to facilitate detection of IPSPs.

Stimulation of ipsilateral ponto-medullary sites 1-2 mm from the midline evoked EPSPs and IPSPs in neck extensor and flexor motoneurons. These PSPs survived coagulation of the medial longitudinal fasciculus (MLF) caudal to the stimulus points and must therefore have been mediated by reticulospinal pathways outside the MLF. Both EPSPs and IPSPs had latencies ranging from 0.7 to 1.5 msec from the stimulus or from 0.1 to 0.9 msec from arrival of the earliest descending volley indicating that both pathways are monosynaptic. These PSPs will be referred to as "RS PSPs".

Stimuli applied near the MLF also evoked monosynaptic EPSPs and IPSPs in neck motoneurons that resembled previously described vestibulospinal and reticulospinal PSPs (4). These PSPs did not occlude the RS PSPs evoked from more lateral sites when medial and lateral stimuli were applied together, which indicates that the two pathways are independent. Monosynaptic PSPs were seldom evoked when points in the contralateral reticular formation or points more than 2 mm from the midline on the ipsilateral side were stimulated.

RS IPSPs were evoked only from nucleus reticularis (n.r.) ventralis and not from more rostral areas. They were observed in 20/27 (74%) neck extensor motoneurons but in only 1/6 (17%) flexor motoneurons. RS EPSPs were evoked from n.r. pontis caudalis, gigantocellularis and ventralis. The EPSP amplitude increased as the stimulus site shifted from anterior to posterior indicating that additional excitatory fibers join the reticulospinal pathway at medullary levels. RS EPSPs were observed in 58/64 (91%) extensor and 11/14 (79%) flexor motoneurons studied. They were significantly larger in extensor motoneurons.

These results indicate that reticulospinal projections to neck motoneurons differ from those to hindlimb motoneurons (1,2,3). Neck motoneurons receive direct excitation and inhibition from the lateral part of the medial ponto-medullary reticular formation whereas hindlimb motoneurons receive excitation only from fibers running in or close to the MLF (1) and apparently do not receive direct reticulospinal inhibition (2,3). It is therefore possible that the lateral parts of the medial reticular nuclei are the source of specialized excitatory and inhibitory reticulospinal projections concerned with controlling head position.

Supported in part by NSF grant BMS 75-00487 and NIH grant NS 02619.

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757 COMPARATIVE STUDY OF MOTOR POTENTIAL IN ANIMAL AND MAN.

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The motor potential is a slow voltage change (1 or more secs.) which can be recorded from the scalp or brain's surface during a voluntary self-paced movement (e.g. hand closure). The response preceeds the onset of movement and is reminiscent of the activity of some motor cortical neurons that occurs with a learned movement in monkey (Evarts). One advantage of recording motor potentials rather than units is that records can be made simultaneously from multiple cortical areas to determine: (1) the cortical regions that become active during a specific movement; (2) the time sequence in which the areas are activated. Such information is important for elucidating cortical mechanisms involved in initiation of movement. Transcortical recording was employed in monkey. In man, recordings were made during operations for epilepsy and the recording electrodes are the same surface electrodes as those used to localize the epileptogenic focus. In monkey it appears that only the motor and somatosensory area generate a response. With the exception of electrically inexcitable (for producing movement) regions in area 4 the remainder of the lateral cortical mantel shows no response during self-paced hand closure. In man the response to a similar movement has a wider distribution with a large response also appearing in area 6 (necessary controls assured that the responses were locally generated and not field potentials of distant origin). In both animal and man a small or no response is seen with ipsilateral hand closure. Also in both, the response in motor cortex begins prior to onset of movement while in the somatosensory area it and movement begins almost simultaneously. In man the sequence of response appearance is - motor cortex, area 6, and somatosensory area. Whether the difference in response distribution between animal and man reflects a true species difference or whether the wider distribution in man is related to the increased excitability of epileptogenic cortex is currently under study.

758 VIBRATORY OCCLUSION OF COMPENSATION FOR MUSCLE YIELDING. W.Z.Rymer, J.C. Houk and P.E. Crago, The Johns Hopkins University School of Medicine, Baltimore, Maryland, 21205. (Supported by NIH 06828 and NIH 57240.)

When an electrically stimulated muscle is stretched, muscle force rises sharply, yields once stretch exceeds a fraction of a millimeter, and sags even further when the dynamic phase of stretch is completed. Stretch reflex mechanisms provide important compensation for yielding (1), but the contribution of different muscle afferents is not known. We have studied the mechanisms of this compensatory action using small amplitude longitudinal tendon vibration to occlude the actions of primary spindle receptors and Golgi tendon organs in the soleus muscle of the decerebrate cat. Ramp muscle stretch was applied via a translational table capable of controlled displacement at constant velocity. Tendon vibration was introduced by a small vibrator attached to the translational table. Muscle tension and vibration amplitude were measured by a set of strain gauges which were incorporated in the beam to which the soleus was attached. We used vibration frequencies of 180-200 hertz, at amplitudes of 100-150 microns. The discharge patterns of 1-4 muscle receptors were monitored simultaneously by recording from fine filaments teased from otherwise intact L7 and S1 dorsal roots. Amplitudes of vibration which phase locked primary ending discharge frequently phase locked Golgi tendon organs as well, but did not affect secondary ending discharge appreciably. The frequency of the phase locked tendon organ discharge was usually a subharmonic of the vibration frequency, although one to one discharge was observed occasionally at high forces. When ramp and plateau stretch were superimposed upon longitudinal tendon vibration, compensation for muscle yielding was poor, producing an initial sequence of force changes approaching the response of electrically activated (areflexic) muscle. These observations are illustrated in the accompanying figure, in which the reflex response to a 2mm soleus stretch is superimposed upon the response to a similar stretch applied in combination with longitudinal tendon vibration. The initial portions of each force trace are closely matched, but the vibration record demonstrates abrupt yielding, once stretch amplitude exceeds 0.2 mm. These results suggest that the reflex effects of primary ending discharge serve to prevent muscle yielding in the normal stretch reflex. When the force response to combined stretch and vibration is compared with that produced by stretch of an electrically activated muscle, the vibrated muscle usually shows a substantially greater response, which develops after ramp completion, and which increases over a period of several seconds. Since primary ending discharge is phase locked by vibration, and tendon organ discharge causes inhibition of extensors, the additional excitation reported is probably an excitatory effect of secondary endings. This result suggests that the later portions of the stretch reflex response include a substantial excitatory contribution from secondary endings, supporting Matthews' recent hypothesis (2).

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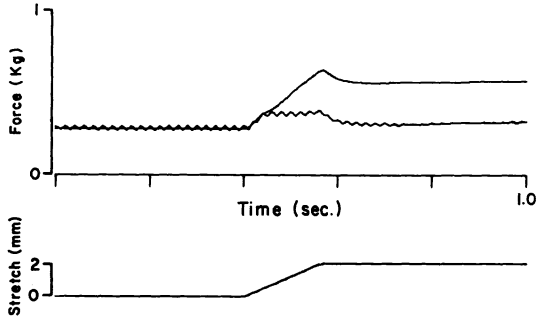

759 THE POSTNATAL MORPHOGENESIS OF THE CAT RED NUCLEUS. Alfredo A. Sadun. Dept. of Neuroscience, Albert Einstein Col. of Med., Bronx, NY 10461.

Kittens (20 for Golgi, 40 for electron microscopy) of varying ages were sacrificed and prepared for studies of the red nucleus (RN). At birth, the RN is linearly 43% of adult size, but only 9% by volume. The RN continues to grow rapidly: at age 3 days, 10% by volume; at 1 week, 12%; at 2 weeks, 16% the volume of the adult RN.

Electron microscopy revealed the myelinization of axons in the RN beginning just before the kitten is 1 week old. After the third week there is little appreciable increase in the thickness of the myelin sheaths. Kittens 3 weeks and younger show many more dendritic spines and undulating varicose dendrites than adult RN tissue. The long dendritic spines are even more readily apparent in kittens less than 1 week of age. Granules which look like lipofuscin are often seen in the magnocellular neurons of kittens as young as 1 week of age.

Sections stained by the Golgi-Cox method were analyzed and four basic cell types were distinguishable on the basis of size, somatic shape, number of primary dendrites, extent and direction of dendritic arborization, nature of dendritic spines, axonal origin, orientation and axonal collaterals. A class of cells, emcompassing the principal magnocellular neurons usually has a single, non-bifurcating axon emerging from the soma in a dorsal direction. In the 3-day kitten RN, the axons from these cells proceed dorsally for 40-60um, then turn medially abruptly and continue in a ventro-medial direction. After the first 50 or 60um, the axons also begin to shift caudally, but they do not turn sharply posteriorly until 100-250um further.

The different cellular types are not distributed evenly along the rostro-caudal axis, nor are the cellular types equal in distribution at the various ages studied. Moreover, there is a striking cellular morphological maturation evident after only a very short postnatal period. The spines of the magnocellular neurons are far more numerous, longer and thinner in the 1-day kitten than in the 3-day animal.

The magnocellular class of neurons is located predominantly in the more caudal portion of the RN. Only very few of these cells show an extensive or preponderant rostrally directed dendritic arborization. Since cortical afferents to the RN are primarily distributed in the rostral regions (Sadun, Brain Research, 99(1) 1975), the magnocellular neurons, which give rise to most, if not all of the rubrospinal fibers, are largely under cerebellar influence and not direct cortical control.

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760 RECRUITMENT OF THE CAT SOLEUS AND LATERAL GASTROCNEMIUS DURING STANDING, STEPPING AND JUMPING. Judith L. Smith, V. Reggie Edgerton and Bill Betts*
Dept. Kinesiology and Brain Res. Inst., UCLA, Los Angeles, Ca. 90024

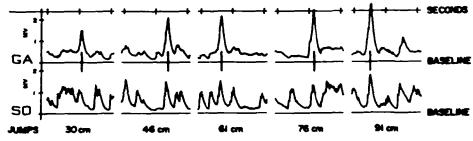
Myopotentials from two ankle extensors, the slow contracting soleus (SOL) and the fast contracting lateral gastrocnemius (LG) have been telemetered from five freely-moving cats. The cats were trained by food deprivation to run on a treadmill at speeds ranging from .5 to 3 m/sec and to jump vertically to a platform placed upwards from 30 to 90 cm. Electromyograms were synchronized with the cat's movements by a video system, which employed two cameras and a special effects generator to record and display both images on a single monitor. In addition, the EMGs were recorded on FM tape for subsequent integration (I-EMG) and computer analyses.

With the cats gently restrained in a prone position, single motor units from both muscles could be identified. During quadrupedal standing, the SOL exhibited greater recruitment than the LG, which was relatively quiet during postural activities until bipedal hindlimb stands were performed.

Both muscles were active just prior to and during the stance phase of treadmill stepping. As locomotion progressed from a slow walk (.5 m/sec) to a slow gallop (2 m/sec), recruitment of LG units, judged by mean peak I-EMG values, increased by a factor of 4 relative to the SOL. During stepping the SOL reached its peak I-EMG activity 40-60 msec before the LG.

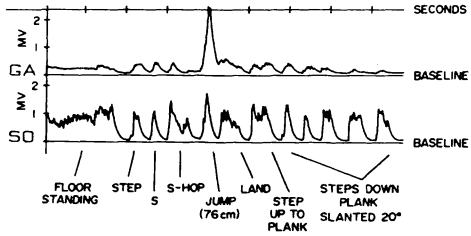
During jumping, both muscles were active with the same temporal asynchrony seen in locomotion. At heights over 46 cm, recruitment of LG units was particularly impressive. I-EMGs plotted for 5 platform jumps recorded in sequence are shown in Fig. 1. Vertical bars indicate the jumps. Each I-EMG signal from the LG (GA) peaked dramatically above adjacent signals, while jump signals from the SOL were difficult to distinguish from the surrounding step, standing and landing signals. As jumps increased from 30 to 90 cm, peak I-EMGs increased, on the average, 3.3 times for LG and only 1.5 times for SOL.

Fig. 1:



A continuous I-EMG record, which shows standing, stepping, hopping and jumping, summarizes our findings (Fig. 2). During standing and floor stepping, SOL units are very active; recruitment increased only minimally as the movement kinetics accelerated. Conversely, recruitment of LG units were greatly influenced by the dynamics of the movement. Both the predominantly fast fiber composition and the mechanical properties of LG units are well suited for the rapid, powerful movements in which the muscle was found to participate. Supported by USPHS Grant (NS10423-03).

Fig. 2:



761 CEREBELLAR NEURON RESPONSE TO IMPOSED LIMB DISPLACEMENT: DEPENDENCE OF SHORT LATENCY DENTATE ACTIVITY ON INTENDED MOVEMENT. Peter L. Strick. Lab. Neurophysiol., NIMH, Bethesda, MD. 20014.

In a prior study of cerebellar activity in the awake monkey (DeLong and Strick) it was observed that the activity of some neurons in the deep cerebellar nuclei could be modified at relatively short latencies by small torque steps delivered to the animal's limb during sustained holding. For some neurons this activity varied markedly with the direction of the imposed displacement, which either flexed or extended the animal's arm. In this task the animal responded to the displacement with an active movement opposing the displacement. Thus, the extent to which the observed changes in neural activity were related to the sensory input evoked by the displacement or to the animal's corrective movement could not be determined. In order to dissociate these two components of cerebellar response to limb displacement, a monkey was trained on the "instruction" paradigm employed by Evarts and Tanji. This task creates a situation in which the direction of the imposed displacement can be dissociated from the direction of the animal's active movement. The monkey was required to grasp a handle and maintain it in a certain position for 2-4 sec; he was then given an instruction how to respond to a torque motor-imposed displacement of the handle. The instruction was a red or green lamp which was illuminated 1.2-2.4 msec prior to the displacement. The red lamp signaled the monkey to push and the green lamp to pull the handle, when he felt it move. The torque motor could impose displacements which moved the handle towards or away from the monkey. Thus, depending on the instruction, an imposed displacement of one direction could trigger movements in either direction, and the imposed displacement would in some cases assist and in others oppose the animal's active movement. Short latency responses, evoked within 60 msec of the imposed displacement, were observed in 46 of the 98 deep cerebellar neurons which were related to the task. Twenty-five of these neurons were recorded in the interpositus (I) and 21 in the dentate (D). Although there was considerable overlap, the activity changes evoked in I neurons occurred, on the average, before those of D neurons. The earliest change in activity occurred approximately 18 msec after the imposed displacement for an I neuron and 27 msec for a D neuron. More striking than these differences in latency, however, were the differences in the relation of neurons in these two nuclei to the task. Fifty percent of the I neurons, influenced at short latency by the imposed displacement, were strongly affected by the direction of the displacement. For example, the discharge of some I neurons showed a reciprocal relation to the direction of the imposed displacement (increasing their discharge following displacements in one direction and decreasing their discharge following displacements in the opposite direction). Other I neurons showed a differential relation to the direction of the imposed displacement, showing changes more strongly affected by imposed displacements in one direction than the other. In contrast, no D neurons showed short latency changes solely affected by the direction of the imposed displacement. The short latency response for 33% of the D neurons, although triggered by the imposed displacement, was contingent on the animal being prepared to move in a particular direction. Furthermore, for some neurons, imposed displacements in either direction would evoke short latency increases in firing when the animal was prepared to move in one direction and decreases in firing when the animal was prepared to move in the opposite direction. Thus, while the activity of many I neurons relates to the direction of the imposed limb displacement, the activity of many D neurons appears to represent a stimulus-triggered preprogram of the intended active movement.

DeLong, M.R. and Strick, P.L., unpublished observations.
Evarts, E.V. and Tanji, J. (1974), Brain Res. 71: 479-494.

762 VARIABLE POSTURAL RESPONSE OF THE DOG TO VISUAL STIMULI DEPENDING UPON THE ENVIRONMENTAL CONTEXT. Richard E. Talbott and John M. Brookhart. Dept. Physiol., Univ. Ore. Med. Sch., Univ. Ore. Health Sci. Ctr., Portland, OR 97201.

The quietly standing dog will compensate for sinusoidal forward-backward) displacements of the support platform upon which the dog stands. The describing function for the compensatory positional movement in response to such perturbations indicates that the overall postural control system of the dog shares certain features in common with an inverted pendulum which is kept upright by means of a controlled restoring torque generator. The control system operates on the basis of information derived from many sensory pathways other than just the signals of perturbed posture derived from the limbs. In particular, visual field motion supplies the postural controller with information which can enhance or constrain the response to platform motion depending upon the relative motions of the two controlled disturbances. Thus, motion of the visual surround in the same direction as the motion of the support platform (0 degrees relative phase) enhances the overall magnitude of the positional response and decreases the phase lag of the positional response relative to platform position over the range 0.1-0.5 Hz. At a relative phase of -90 degrees between platform and visual surround motion (visual field motion lagging), the dog's positional response also exhibits an enhanced magnitude, but the phase lag with respect to the platform position tends to approximate that seen in the absence of additional visual field motion. Based on the response of the dog to visual field motion alone, it would be natural to predict that the net positional response to combined visual field and platform motion at relative phase of -180 degrees would be less in magnitude than the response to platform motion alone. That prediction is not borne out experimentally. There is a net reduction in the magnitude and an increased phase lag of the positional response to -180 degree mixed stimulation when compared with the response to 0 degree mixed perturbation, but the magnitude of the response to -180 degree relative motion is similar to or slightly greater than the magnitude found with a stationary visual field. Similarly, the positional response with mixed inputs at -180 degrees relative phase exhibits a reduction in lag relative to platform position over the range 0.1-0.5 Hz when compared with the stationary visual field responses. The mismatch between prediction and experiment can partially be accounted for by an increase in gain for the visual input channel during mixed perturbation. Previously (Talbott, 1975, Physiologist 18(3):416) an explicit model utilizing visual feedback led to the expectation that the visual signal must be processed at a higher gain during platform perturbation than when visual field motion alone was the perturbing stimulus. Additional support for this concept of variable gain depending on the environmental context is provided by positional responses to mixed stimulation but with the two inputs at different frequencies. Thus, if visual field motion is at 0.3 Hz and platform motion is at 1.087 Hz, the 0.3 Hz component of the dog's positional response is more than 10 times what would be expected on the basis of the response to a 0.3 Hz visual field motion in isolation. Any 'noise' at 0.3 Hz in the dog's response to platform motion alone at 1.087 Hz is a reflection of the non-linear nature of the postural control system; the magnitude and phase of such non-linear response components will not explain the excess or increase in the 0.3 Hz component found when visual field motion at that frequency is present. The goal for future experiments is to tease out the mechanisms underlying this ability of the postural control system to selectively modulate the sensitivity of response to various sensory inputs.

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763 EFFECT OF PRENATAL FORELIMB DEAFFERENTATION TWO-THIRDS THROUGH GESTATION ON MOVEMENTS DURING SECOND YEAR OF LIFE IN MONKEYS. Edward Taub, Robert D. Heitmann*, H. Cannon Grier*, and Gilbert Barro*. Inst. for Behavioral Research, Silver Spring, MD. 20910.

Rhesus monkey fetuses were exteriorized at the end of the second trimester of pregnancy, placed in a temperature-controlled saline bath, subjected to forelimb deafferentation (C_1 or C_2-T_4), and then replaced in utero for completion of fetal development. In previous work, the uncalcified pedicles of the fetal vertebrae had collapsed and spread laterally following removal of the laminae. The spinal cord, left unprotected, suffered severe injury due to compression by overlying muscles. After birth the animals exhibited marked quadriparesis. In order to protect the spinal cord from this type of injury, a vertebral prosthesis was developed to substitute for the portions of bone removed during surgery. This device was then emplaced when fetuses were given left forelimb deafferentation two-thirds through gestation. Two infants were recovered live at Caesarian section and have survived into the middle of the second year of life (at the time of this writing).

The original prosthesis was rigid along its long axis. In one animal it has been left in situ until the present, while in the other animal it was replaced at 3 months by a segmented prosthesis which affords considerable flexibility. X-rays reveal that the prostheses have not shifted since time of emplacement. Hindlimb function is apparently normal in both animals indicating that the prostheses have been successful in providing the spinal cord long-term protection from serious injury.

During the first year of life both animals exhibited an extensive repertoire of behavior with the deafferented limb. It was used for postural support during standing and sitting, ambulation (with placement predominantly on the wrist dorsum), and reaching toward objects. The hand could be cupped around ropes and ladder rungs in climbing; however, the ability to grasp small objects between thumb and forefinger did not develop spontaneously. Attempts were begun to improve manual dexterity in one infant at the age of 6 months and in the other infant at 1 year by means of gradual "shaping" procedures involving a series of steps of progressively increasing performance difficulty. Both infants developed the ability to pick up small food objects between thumb and forefinger, and one of them can now secure the food objects from shallow wells on a dexterity board. For this animal, training resulted in an improvement in use of the deafferented hand in the free situation. During ambulation, placement of the hand is now exclusively on the palmar surface.

Intrauterine surgery provides a solution to the problem of the inaccessibility of the mammalian fetus and makes possible a new approach to the study of the development of the nervous system and behavior. The results from this experiment indicate that somatosensory feedback and local spinal reflexes are not necessary in monkeys during the final third of pregnancy for the development of many types of forelimb movements. This finding suggests that the motor programs for many movement patterns may be endogenous.

(Supported by NIH Grants HD 08579 and FR 5501RR05636.)

764 LIMITATIONS OF VOLITIONAL CONTROL OF SINGLE MOTOR UNIT RECRUITMENT SEQUENCE J. S. Thomas, E. M. Schmidt, and F. T. Hambrecht, Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

Previous studies have suggested that single motor unit firing can be volitionally separated from the pattern seen during spontaneous recruitment in the production of muscle force, provided the appropriate feedback signals are made available to the subject. During the course of experiments designed to test the practicality of using single unit firing frequency as a signal for control of orthotic devices we have been similarly impressed by the apparent ease with which the firing patterns of "isolated" single motor units can be controlled. However, careful monitoring of motor unit potential waveform and surface recorded EMG activity has led us to several conclusions which suggest that voluntary motor unit activation does not differ from normal volitional control of fine movements. Specifically: 1) Activity that can be recorded as isolated unit spikes on the paired wire, intramuscular, electrodes "classically" used in such experiments (Basmajian, J.V. and Stecko, G., J. Applied Physiol. 17, 849, 1962) are typically among the first units spontaneously recruited and are therefore confined to a very narrow range of spontaneous recruitment level. 2) Such electrodes are more selective than surface EMG electrodes and do not effectively monitor total activity in the muscle thus simplifying the process of "isolation". 3) Variations in digit, wrist, or limb position are often effective in altering the waveform of the "same" motor unit recorded with these electrodes. 4) Using somewhat more stable and selective electrodes we were able to isolate units (identified by paired intramuscular electrode arrays and surface EMG waveform) through a wide range of volitional force in various arm and hand muscles. Clear reversal of recruitment order of separate motor units recorded from the same electrode was effected only in muscles whose geometry might allow interdigitated motor units to subserve markedly different directions of contraction (e.g., abductor pollicis brevis). Such unit control was always accomplished by willing contractions of very different orientation (or of different fingers in the case of extensor digitorum). Thus, previous findings of volitional control of motor unit recruitment order would not appear to involve the mediation of "motor unit specific" volitional intervention but rather to reflect either the spontaneous activation of adjacent or interdigitated motor units, each activated by volitional effort along its distinct kinesiologic axis, or to devolve from volitional alterations in recording geometry.

The ease and precision of volitional control of motor unit firing frequency was tested by means of an 8 increment frequency tracking task, the span of which was adjusted to cover the full range of discriminable frequency modulation. Comparison of performance on this "single unit task" with that achieved under comparable conditions by the rectified surface EMG signal over the same range of volitional effort showed surface EMG provided a better control signal than single unit activity.

765 BRANCHING OF CORTICOSPINAL FIBERS IN THE MONKEY. H. Asanuma, Y. Shinoda* and P. Zarzecki. The Rockefeller University, New York, N.Y. 10021.

The intraspinal spread of axon branches of corticospinal (CS) neurons was examined. Several glass insulated tungsten microelectrodes were implanted into the cervical gray matter and a larger electrode into the lateral corticospinal tract at an upper thoracic level. CS neurons in the motor cortex were identified by antidromic responses to microstimulation of cervical gray matter. In addition, supramaximal stimulation was delivered through the thoracic electrode to determine whether the same neurons sent axons to a lower level. Collision techniques were used to ascertain that the separate stimuli activated different axon branches of the same neuron.

A total of 235 CS neurons were activated by stimulation of cervical gray matter and 56 of them were activated from motoneuron pools. About 30% of each group were activated not only from the cervical gray matter, but also from the thoracic corticospinal tract. The rest of the neurons (70%) were activated only from the cervical cord and the majority of these (85%) were activated by one or two electrodes located within one cervical segment. It is concluded that single CS neurons may influence the activity of separate motoneuron pools. (Supported by NIH grant # NS-10705).

766 A CIRCULATORY HYPOTHESIS FOR STEADY STATE MAGNETIC FIELD EFFECTS ON MUSCLE. Richard A. Berg and Douglas L. Chute. Dept. of Psych., Univ. of Houston, Houston, Tx. 77004.

Magnetic fields have been shown to affect contractility of striate muscle (Reid, 1972; Bucking, Herbst, and Piontek, 1974). In our first experiment, similar results were obtained where human grip strength showed a significant ($p < .05$) decrease. In two later experiments to determine the mechanism of the field effect, we observed that circulation in the presence of a 150 gauss steady state magnetic field was the probable mechanism of action. Using the measurement techniques described by Mailman and Jordan (1975), the first experiment measured Na absorption, total blood flow, and regional blood flow in canine ileum. A decrease in flow in the presence of the magnetic field was shown on all measures, with a 29% decrease of regional blood flow in the villae being the largest ($p < .0001$). The second experiment used human subjects where the presence of the magnetic field significantly ($p < .0001$) lowered by 33% cold pressor induced muscle tension measured in integrated millivolt/minute EMG. In both experiments, an equivalent "dummy magnet" was counterbalanced with the magnet conditions. In concordance with Reid (1972) the results are interpreted as a circulatory mechanism for the clinical findings in humans that steady state magnetic fields can relieve the symptoms of systremma; severe "calf" muscle cramping.

767 CORTICAL PROJECTIONS TO CERVICAL AND LUMBAR SEGMENTS OF SPINAL CORD IN THE RHESUS MONKEY USING THE RETROGRADE TRANSPORT METHOD. Michael P. Biber,* Lawrence W. Kneisley,* Jennifer H. LaVail and Pasko Rakic. Depts. of Neuropathology, Harvard Medical School and Neuroscience, The Children's Hospital Medical Center, Boston, MA 02115.

The distribution of cell bodies of corticospinal axons that project to cervical and lumbar segments of the spinal cord of rhesus monkeys has been determined using the retrograde transport method. Multiple unilateral injections of horseradish peroxidase (HRP) were made in the cervical or lumbar enlargement of the spinal cord of 4 animals. Forty-eight hrs after cervical and 72 hrs after lumbar injections, the monkeys were perfused with a combined aldehyde fixative, and sections of brain and cord were reacted with diaminobenzidine and H_2O_2. In the one lumbar preparation additional sections were reacted with benzidine according to the protocol of Mesulam (1976). In all animals HRP-labeled cells were restricted to cortical layer V, ranged in size from medium (220 μm^2) to large (1800 μm^2) pyramids, and were confined to areas 4, 1, 2, 3, and 5 of Brodmann. The greatest concentration of cells was consistently found in area 4, even in those cases in which injections by-passed the lateral funiculus and entered the anterior and posterior horns of the cord via the dorsal funiculus. The labeled cortical neurons were located primarily contralaterally, even though the injected HRP diffused into the opposite hemicord in some cases. HRP-positive neurons in the lumbar experiment were most concentrated dorsally on the convexity and on the medial surface of area 4. Labeled cells in the cervical experiment were most concentrated in the lateral aspect of area 4, but also were found medially; their distribution overlapped that of the lumbar experiment. (Supported in part by N.I.H. grants 11233, 12527 and by the Research Associate Investigator Program, V.A. Hospital, West Roxbury, MA.)

768 CONSISTENCY AND SENSITIVITY OF TENDON ORGAN RESPONSES TO REPETITIVE STIMULATION OF SINGLE MOTOR UNITS. M. D. Binder*, J. S. Kroin*, G. P. Moore and D. G. Stuart. Dept. of Physiol. Univ. of Arizona Med. School, Tucson, Az. 85724 and Dept. of Biomedical Engineering, Univ. of Southern California, Los Angeles, Ca. 90007.

It has been previously demonstrated that a single motor unit contraction constitutes an effective stimulus to Golgi tendon organs. We have used crosscorrelation analysis to quantitate and compare the responses of cat soleus tendon organs to; (1) the contractions of different motor units within the muscle, (2) the contractions of a single motor unit at different muscle lengths, and (3) the contractions of a single motor unit when the pulse-train pattern delivered to the motor unit axon was altered. Our results indicate that the response of a tendon organ to repetitive stimulation of a single motor unit is remarkably consistent with respect to both the number of spikes and the time of occurrence of each afferent discharge. In a typical experimental run involving several hundred motor unit contractions, single Ib afferents have been observed which always responded with the same number of spikes (usually one to four) and with post-stimulus latencies not differing by more than 1-2%. In addition, the crosscorrelation histograms reveal that tendon organs are sensitive to the subtle changes in twitch waveform and amplitude which occur in an average motor unit contraction when the muscle length and/or pattern of motor unit discharge are varied. (Supported in part by USPHS grants NS 07888 and NS 11298 and the Fan Kane Foundation)

769 EVIDENCE FOR SELECTIVE INPUTS TO A MOTONEURON POOL. H.P. Clamann and C.G. Kukulka*. Dept. of Physiol., Med. Coll. of Virginia, Richmond, VA. 23298.

Intracellular recordings were made from spinal motoneurons in chloralose-anesthetized cats. Impaled cells were identified as medial gastrocnemius (M.G.) motoneurons by antidromic stimulation of the M.G. muscle nerve. The critical firing level (C.F.L.) of an M.G. motoneuron was determined by stimulating L7 and S1 dorsal roots (D.R.) while simultaneously recording the response of the motoneuron and the magnitude of the monosynaptic response in the M.G. muscle nerve. Stimulation was then applied to either L7 or S1 D.R. alone and the C.F.L. of the cell was again measured. Finally, C.F.L. was re-tested in response to L7 + S1 D.R. stimulation. Only M.G. motoneurons whose C.F.L. in response to L7 + S1 D.R. stimulation did not change with time were considered for study.

More than two-thirds of M.G. motoneurons showed a different C.F.L. in response to L7 + S1 D.R. stimulation than to stimulation of one D.R. alone. Stimulation of S1 D.R. will activate a population consisting predominantly of M.G. spindle afferents while the population of lateral gastrocnemius and soleus (L.G.-S.) spindle afferents is concentrated in L7. It is suggested that heteronymous inputs, perhaps from L.G.-S., may be directed to a sub-population of M.G. motoneurons of similar size. It is known that L.G.-S. spindle afferents make monosynaptic connections with only 60% of M.G. motoneurons. Selective excitation of such a sub-population would cause the C.F.L. of cells receiving the input to fall relative to that of other cells in the M.G. pool.

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770 Long-loop reflexes in the tranquilized monkey. J.D. Cooke and M.J. Eastman*, Dept. of Physiology, Univ. of Western Ontario, London, Ontario

Recent studies have indicated that psychological 'set' or expectancy as well as learning may be involved in the control of long-latency reflex responses to limb perturbation. We have recorded EMGs from forearm flexors in two tranquilized Cebus monkeys in which these factors were presumably absent. The EMGs showed three distinct peaks in response to sudden arm displacement. These responses, which also occurred in two completely 'naive' monkeys, occurred at latencies corresponding to the M1, M2 and M3 reflex responses seen in the alert primate and human. Thus, although such things as set, expectancy and learning may modify long-latency reflex activity, they are not absolute prerequisites for such activity.

The magnitude of all three responses increased with increasing background activity. M1 and M2 responses were position dependent, M1 being larger in extension than in flexion and M2 the reverse. A dependence on background and arm position was also found in the forearm extensors in which M1 was greater in flexion and M2 in extension. A period of depressed EMG activity lasting for 25 - 40 msec following M1 was dependent on the M1 magnitude. It was found that the apparent M2 position dependency could be accounted for by an M1-dependent depression of M2 reflex activity in the period following M1.

Supported in part by the Medical Research Council of Canada (MT-4465) and the U.S. Public Health Service (NS-10311).

771 THE EFFECTS OF PARTIAL, PRIMARY DEAFFERENTATION ON CONTRACTILITY AND DYSTONIA OF DYSTROPHIC MOUSE MUSCLE (C57BL/6J dy^{2J}/dy^{2J}).
W. B. Douglas. Dept. Neurosciences, McMaster Univ. Med. Centre, Hamilton, Ontario, Canada L8S 4J9.

Myographic recordings of dystonic contractions of anterior tibialis of dystrophic mice (2J allele) suggest an important determinant of the spontaneous muscle contractions may be afferent activity from muscle stretch receptors. A primary trait of the mutant mouse 'Sprawling' (Swl/+) is a 95% decrease of the numbers of muscle spindles in hind leg muscles of affected mice [Duchen, L. W. (1975) J. Neuropath. Appl. Neurobiol. 1: 89.]. Breeding of Swl/+ $dy^{2J}/+$ ♀ X +/+ dy^{2J}/dy^{2J} ♂ yields offspring which are sprawling and dystrophic, Swl/+ dy^{2J}/dy^{2J}. The synergistic effects of the interaction of a primary afferent anomaly with a primary myopathy are: 1) reduced growth rate; 2) clinical expression of dystrophy at 2 postnatal days of age; 3) isometric twitch and tetanic tensions 2/3 of +/+ dy^{2J}/dy^{2J} dystrophic controls; and 4) cessation of dystonic contractions of dystrophic muscle. Apparently, dystrophic mouse muscles, when deprived developmentally of their normal complement of stretch receptors, undergo an increased rate of degeneration. The results imply that the dystonia of murine dystrophic muscle, expressed through the activity of muscle afferents, has a "conditioning" effect on developing dystrophic muscle which retards the myopathic degeneration of extrafusal fibers characteristic of murine muscular dystrophy.
(This study is supported by a Research Fellowship from the Muscular Dystrophy Association of Canada.)

772 TERMINAL DISTRIBUTION AND RESPONSE PROPERTIES OF CORTICOMOTONEURONAL CELLS IN ALERT MONKEYS. E. E. Fetz and P. D. Cheney. Dept. Physiol. & Biophysics and Reg. Primate Res. Ctr., University of Washington, Seattle, WA 98195.

In monkeys trained to alternately flex and extend the wrist against programmed loads we recorded activity of covarying precentral cortex cells and up to 12 forelimb muscles with indwelling electrodes. To detect the existence and extent of corticomotoneuronal (CM) connections, post-spike averages of rectified EMG activity of 5-6 covarying wrist muscles were computed for 135 task-related cells (min. average of 2000 events). The action potentials of 50 cells were followed by a transient post-spike facilitation (PSF) in the average EMG of one or more muscles. The latency and time course of such PSF suggest they were mediated by monosynaptic CM connections. 32 cells were followed by PSF in two muscles (20) or more (12). Cross-correlating muscle activity confirmed that EMG electrodes had recorded independent motor units; cross-correlating strongly covarying cortical cells revealed no spike synchronization. We conclude that the cells with PSF in more than one muscle distributed CM terminals to the associated motoneuron pools.

Activity of CM cells usually covaried closely with the phasic and tonic activity of muscles exhibiting PSF. Most CM cells became active 20-60 msec prior to EMG onset. Response averages compiled separately for movements against different loads revealed that during hold periods the average tonic firing rate of many CM cells covaried linearly with maintained force. Below this linear range some cells exhibited constant rates; others were inactive below a threshold force level. This suggests that both increases in firing rate and recruitment of CM cells may contribute to generation of static force.

773 EMG PATTERNS AND FORCES DEVELOPED DURING STEP-DOWN. William Freedman and Richard Herman. Krusen Research Center, Temple Univ., Phila., Pa. 19141.

These experiments describe electromyographic activity and forces developed during step-down from several heights. The external environment of the experimental subjects was varied by blindfold or Achilles' tendon vibration during descent. EMG activity of the step-down limb, vertical force of impact and knee angle were measured. The results show that the triceps surae muscle contracts prior to the time of impact of the foot with the substrate if information about the step height is supplied to the subject and if the sensory system is not disturbed by vibration. In the presence of vibration or if the step height is unknown, the triceps surae of many people does not contract prior to foot contact. This produces a large force transient which must be absorbed by the skeletal structure, rather than being damped by a lengthening contraction of the triceps surae. The data suggest that the forces developed during ordinary step-down may be the cause of bone and joint injury in people with impaired vision or in patients with damaged nervous systems who exhibit large responses to vibration.

774 PLASTICITY IN THE VENTROLATERAL THALAMUS. T.L.Frigyesi. Depts. Physiology, Texas Tech U.Sch.Med. 79409; and N.Y.Medical College, Valhalla, N.Y. 10595.

Several monosynaptic input-systems to the ventrolateral thalamic nucleus (VL) have been identified by combined neuroanatomical and electrophysiological methods. It was observed in encephale isole as well as penthrane anesthetized cats and squirrel and cebus monkeys that under specific conditions evoked monosynaptic EPSPs (and extracellular unit discharges or negative focal potentials) in VL were first selectively abolished and, then, stimulation of the same electrodes with identical parameters evoked, at the same recording sites, excitatory responses which exhibited similar electrographic properties but longer latencies and durations than in the control states. The monosynaptic input-systems to VL which underwent these alterations were those which arose in the deep cerebellar nuclei, motor cortex and caudate nucleus. The experimental conditions effecting these alterations were as follows: penicillin focus in the motor cortex induced such changes in the brachium conjunctivum-VL-motor cortex projection system; systemic administration of L-DOPA induced them in the motor cortex-VL projections; anticholinergic drugs induced them in the cerebello-VL pathway; and tetanization of the caudate nucleus induced them in the caudate-VL projections. Motor cortex and medial thalamus evoked EPSP-IPSP sequences in VL (in part mediated by the thalamic reticular nucleus) were similarly affected by L-DOPA. The phenomena were essentially similar, but not identical, in cats and monkeys. The data show that, under specific conditions, the VL behaves as if its constituent neurons have changed some of their connections. It is postulated that intra-VL, dendro-dendritic, closed chains (akin to the short-axoned neuronal chains elsewhere in the thalamus) which were operative in the immature state of the animals, and whose operations were suppressed during ontogenesis, were reactivated.

775 RELATION BETWEEN ACTIVITY IN RED NUCLEUS NEURONS AND FORCE IN A TRACKING TASK IN THE CAT. C. Ghez and K. Kubota*. The Rockefeller University, New York, N.Y. 10021.

Cats were trained to accurately position a lever with their forearm in relation to a compensatory visual display. The target position was stepped at random times following an initial period of alignment and food given after subsequent realignment. Movement of the forelimb was restricted to the forearm and extension had to overcome simulated springs of variable stiffness. Latencies from target perturbation to initial force change associated with movement were regularly about 100 msec. At movement initiation cocontraction of agonist and antagonist muscles preceded force change by about 20 msec.

The activity of neurons in red nucleus was monitored during task performance to determine timing, patterns of activity and relationship to extensor force. Rubral neurons were observed whose firing frequency was modulated in association with contralateral forearm movements. With rare exceptions the change in firing frequency occurred prior to the force change associated with movement (mean about 40 msec) and was maximal prior to peak rate of force change, then declined. These units showed marked dynamic features. Peak frequencies correlated with maximal rate of force change. The discharge frequency of some of these units was also correlated with the force exerted during maintained posture. In some units a brief pause interrupted the burst of activity after movement initiation. This pause coincided roughly with peak force and peak velocity. The observations suggest that rubral neurons contribute to force generation. The dynamic characteristics, similar to those previously noted in monkey motor cortex may similarly help overcome the 'low pass' properties of muscle. (Supported by NIH grant # NS-10705).

776 THE FINE STRUCTURE OF DEGENERATING CORTICOSPINAL TRACT (CST) TERMINALS IN THE LATERAL BASAL REGION (LBR) OF THE CERVICAL SPINAL CORD OF THE CAT. J. Hanaway and J. Smith. Department of Neurology, Washington University, St. Louis, Missouri 63110.

The purpose of this study was to demonstrate the type, density and distribution of degenerating CST terminals in the LBR (lateral half of Rexed's laminae V & VI) of the cat cervical spinal cord. Cats used in this study underwent frontal leukotomies and were sacrificed from 36 hrs to 7 days after surgery. Intra-aortic perfusion fixation was performed with 3 liters of 1.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate buffer. Degenerating terminals were first seen as early as 36 hrs. Maximum degeneration was at day 3 and did not appreciably change up to day 7. The predominant type of degeneration was the filamentous type. Cystic degeneration, of terminals was also seen. Dark degeneration, described by others was not seen. The distribution of degenerating terminals was predominantly on distal and proximal dendrites and rarely on the soma. An average of 1800 terminals were counted for each time of sacrifice and we found 4.2% degenerations at 36 hrs, 9.6% at 2 days, 11.9% at 3 days, 6.9% at 4 days, and 10.2% at 7 days. Preliminary counts in the medial half of the base of the dorsal horn have shown less than half the percentage of degenerating terminals seen in the LBR. The concentration of degenerating CST terminals determined by electron microscopy in the LBR is much less than the impression one gets from similar light microscopic studies.

777 THE CONTRIBUTION OF NEOCORTEX TO MOVEMENT IN A PRIMITIVE MAMMAL. Rickye Heffner and Bruce Masterton. Dept. Psychology, Florida State University, Tallahassee, FL 32306.

The corticospinal system shows wide anatomical and functional variation among mammals. In Primates it is a relatively large system that contributes greatly to movements of the distal musculature. However, in neurological relics, such as opossums and hedghogs, its transection results in deficits much less dramatic and often escaping detection. In order to investigate the role of a primitive form of this system, cortical and pyramidal lesions were made in hedgehogs and a wide variety of behavioral tests administered. The results show that routine clinical tests for placing, muscle tone, paresis, etc., were entirely unaffected even by complete decortication. However, locomotor deficits could be observed when the subjects were required to walk over difficult terrain. In a hemidecorticate, this deficit was contralateral to the lesion, but in animals with unilateral pyramidal tract sections, the deficit was ipsilateral to the lesion. These results suggest that the corticospinal system of hedgehogs is not entirely crossed even though the net influence of its neocortex is to the contralateral side. A similar deficit was also observed in an animal with damage to the medial lemniscus. In a final series of tests designed to reveal symptoms of release, the animals were required to inhibit a withdrawal from shock to the forepaw. Only those animals with damage to their corticospinal system showed a deficit in their ability to inhibit this spinal reflex. These results suggest that one of the most primitive roles of the corticospinal system may have been to allow the forebrain to overrule spinal cord mechanisms when conflicts between the immediate welfare of the periphery and the long-range welfare of the organism arose.

778 ENDOGENOUS PEROXIDASE IN THE CAUDAL NEURAXIS OF CNS. V.K.S. Iyengar* (SPON: M.M. Rapport). Division of Neuroscience, N. Y. State Psychiatric Institute, New York, N. Y. 10032.

It was recently shown histologically that endogenous peroxidase has a differential distribution in various subdivisions of the rostral neuraxis (telencephalon) of adult rat CNS (Iyengar, 1976). In this region the staining is diffuse and does not permit perikarya to be discerned. In extending these studies to the caudal neuraxis (cerebellum, pons, medulla, cervical cord) it has been found that there are distinct neuronal groups with perikarya showing intense peroxidase activity, which make them clearly distinguishable from the surrounding neuropil. These are: Purkinje cells, deep cerebellar nuclei, locus coeruleus, motor nuclei of the V, VII, and XII cranial nerves, corpus trapezoid nuclei, vestibular complex, reticular formation of the medulla (gigantocellular division?, n. lateralis reticularis?) and a few motor neurons of the cervical cord in the anterior horn. The pattern of endogenous peroxidase distribution in cerebellum, stem, and cord is thus very different from telencephalon. Peroxidase activity appears to be closely associated with neurons controlling and/or co-ordinating motor activities at the spinal and lower supraspinal levels.

779 MOTOR AND SENSORY REGIONS OF THE RHESUS MONKEY VENTRAL THALAMIC NUCLEI DEFINED BY THEIR AFFERENT AND EFFERENT CONNECTIONS. K. Kalil* (SPON: W.I. Welker), Department of Anatomy, University of Wisconsin, Madison, WI 53706.

By tracing their afferent and efferent connections, an attempt was made to define in the rhesus monkey the precise motor and sensory regions of the ventral thalamic nuclei. By injecting small volumes of ^{3}H proline into the ventrolateral (VL) and ventroposterolateral (VPL) nuclei it was possible to define a border zone between these cell groups (localized in the posterior region of VPLo and the rostral extreme of VPLc) which projects exclusively upon area 3a in the floor of the central sulcus. By contrast, injections into rostral VPLo resulted in labeling of motor cortex alone, while those centered in VPLc and caudal VPLo revealed a dense projection to sensory areas exclusively. These results were correlated (often in the same animal) with the thalamic projections of the dorsal column nuclei, traced with either Fink-Heimer or autoradiographic methods. It was found that the DCN project primarily upon the VPLc and that their efferent fibers do not extend beyond the VPLc-VPLo border region which projects upon area 3a. Preliminary autoradiographic results suggest that cerebellar fibers terminate in motor regions rostral to the sensory recipient regions of the ventral nuclear complex. These results show that the motor and sensory regions of the monkey's ventral thalamus are separate and discrete. Thus, physiologically demonstrated peripheral afferent input to the motor cortex must travel by routes other than classical lemniscal pathways.

780 A QUANTITATIVE STUDY OF THE ELECTROMYOGRAPHIC RESPONSE TO SUDDEN ANKLE DISPLACEMENTS IN MAN. R.E. Kearney* and C.W.Y. Chan* (SPON: R. Capek), Biomed. Eng. & Aviation Med. Res. Units, McGill Univ., Montreal, Que, Canada.

The effects of displacement amplitude and ramp velocity on the response of the gastrocnemius (GS) and tibialis anterior (TA) were studied in 5 humans instructed to oppose sudden, servo-controlled ramp displacements of the ankle. In GS, EMG records showed an early monosynaptic response (MSR) to stretch, followed by a silent period, and then a large asynchronous burst responsible for most of the opposing muscle torque (the Functional Stretch Response, FSR). In TA, MSR-FSR components were also noted, but frequently there was an additional, intermediate burst, tentatively termed the "polysynaptic stretch reflex" (PSR). MSR amplitude of both GS and TA increased consistently with ramp velocity (r=0.63,0.53 respectively), but bore an inconsistent relationship with displacement amplitude. The result is consistent with the dynamic sensitivity of muscle spindle primary afferents known to mediate the MSR. Moreover in TA, PSR amplitude tended to decrease with increasing velocity and increase with increasing amplitude. The origin of this behaviour is unclear. Finally, the FSR latency in both muscles bore no significant correlation with velocity (r=0.05), but decreased consistently with increasing amplitude (r=0.65). This finding contradicted Phillip's[1] original proposition of a Group Ia contribution to a transcortical stretch reflex servo-loop thought to be mediating the FSR[2,3,4,5].

1. Phillips, C.G., Proc.Roy.Soc. 173B:141(1969); 2. Chan,C.W.Y., Master Thesis, McGill Univ,(1974); 3. Evarts,E.V., Science, 179:501(1973); 4. Marsden, C.D., et al, Lancet, 1:759(1973); 5. Tatton, W.G., et al, Brain Res. 96:108(1975).

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781 MYOTONIA IN DYSTROPHIC MICE. Peter K. Law*. (SPON:F.R. Freeman) Dept. Neurol., Vanderbilt Univ. Med. Ctr.,Nashville, TN 37232

Coaxial electrode electromyography reveals that myotonia is present in twelve C57BL6/dy^{2J} and three 129ReJ/dy *dystrophic mice examined* at 6 to 10 months ex utero. Both the classical "dive-bomber" myotonic discharges and the pseudomyotonic discharges are present though the latter predominates. Such activity manifests as spontaneous contraction of the trunk and hind-limb skeletal muscles and is most pronounced in the thigh muscles. The fore-limb muscles are relatively spared. Such activity is more prominent in the C57BL6/dy^{2J} strain than in the 129ReJ/dy strain and may be detected in dystrophic mice of the former as early as 3½ weeks ex utero. Discharges from the biceps femoris muscle become intensified when central inhibition is removed from nine dy^{2J}/dy^{2J} mice by spinalizing at the L1 vertebral segment, but disappear completely when the dorsal roots at L4,L5,L6 are severed. Spontaneous action potentials recorded from single nerve fibers at the dorsal roots have shorter latency than the corresponding discharge potentials recorded from muscle fibers. These results are interpreted to indicate that certain sensory receptors in the dystrophic mice are affected and they initiate the spontaneous discharges onto the motoneurones. The motoneurones then bombard on the muscle. (Supported by the Muscular Dystrophy Association of Canada, and the Muscular Dystrophy Association of America, Inc.)

782 SOMASTHETIC UNIT ACTIVITY DURING NORMAL WALKING IN THE CAT. Gerald E. Loeb and William B. Marks. Laboratory of Neural Control, NINCDS, National Institutes of Health, Bethesda, MD 20014

A new technique has been devised which permits simultaneous recordings of unitary extracellular action potentials from cell bodies in the L7 dorsal root ganglion of an unrestrained cat. It has been possible to record from the same afferent unit for periods of hours to days, permitting correlation of patterns of discharge seen during videotaped treadmill walking with studies of receptor characteristics under general anesthesia and controlled mechanical and electrical stimulation. A ten channel "back pack" amplifier relays activity to an FM tape recorder from six floating microelectrodes in the ganglion plus four chronically implanted EMG electrodes.

Preliminary results indicate some firing patterns which might not be anticipated from static testing data; e.g., a light touch receptor in the toe pad had consistent activity only during lift-off and frequently gave bursts during the swing phase; a very rhythmic bursting activity which was not correlated with locomotion or any observed physiological process and persisted during anesthesia was seen in one neuron whose modality could not be determined.

783 ACTIVITY OF TRIGEMINAL MOTONEURONES IN AWAKE MONKEYS DURING PRECISION BITING. J.P. Lund, A. Smith, and B. Sessle. Faculté de Médecine dentaire et Centre de Recherche en Sciences neurologiques, Université de Montréal, and Dental Faculty, University of Toronto.

Monkeys were trained to apply and maintain a specified pressure for a period of 1 second on a force transducer placed between the animal's incisor teeth. Successful trials were rewarded with fruit juice. Indwelling electrodes were implanted in the temporalis, masseter and digastric muscles for recording of electromyographic activity, and around the masseteric nerve for antidromic stimulation of trigeminal motoneurones. Neurones in the trigeminal brain stem nuclei were recorded with microelectrodes. Preliminary results are generally in accordance with previous anatomical and motor unit studies. The trigeminal motor nucleus is topographically organized with the jaw opening muscles represented dorsally and the jaw closing motoneurones ventrally situated. The majority of units encountered were active during jaw closure. They showed marked differences in their force recruitment threshold and firing frequency increased with increasing force levels. Neurones presumed to be γ motoneurones on the basis of their conduction velocity (15-25 m/sec) and collision of antidromic and orthodromic spikes, fired at high frequency during rest, increased their firing rate during the force ramp and maintained it during the plateau phase.

Supported by M.R.C.

784 EFFECT OF SUBTHALAMIC LESIONS ON TREMOR AND SPONTANEOUS RHYTHMIC (5/SEC) THALAMIC MULTIUNIT ACTIVITY IN MONKEYS. Héctor Maldonado*, Francisco Velasco and Marcos Velasco. Sci. Res. Dept. Natl. Med. Ctr. IMSS. Mexico Cy. Mexico.

Present report deals with effect of circumscribed subthalamic lesions on tremor production and arrest and on a spontaneous rhythmic 5/sec multiunit activity from VL thalamic nucleus (VL-MUA) postulated to be related to the pace maker mechanism for peripheral tremor. VL-MUA was recorded within the same monkey, before and after two different sequential subthalamic lesions. The first lesion for tremor production (ventro medial substantia nigra) and the second for tremor blockage (nucleus reticularis mesencephali). A comparative analyses was done in number and frequency of VL-MUA of these various recordings and no significant differences were found prior and after lesions. We conclude therefore that subthalamic lesions for tremor production and arrest are independent to the neurophysiologic mechanism underlying VL-MUA.

785 ALTERATIONS IN THE PHYSIOLOGIC AND METABOLIC PROPERTIES OF CAT TIBIALIS ANTERIOR WITH ELECTRICALLY INDUCED EXERCISE. J. Thomas Mortimer and Uros Roessmann*. Dept. Biomedical Engineering and Inst. of Pathology, Case Western Reserve University, Cleveland, Ohio. 44106

Long-term electrical stimulation applied to the tibialis anterior muscle has been shown to convert the properties of the stimulated region from that of a fast twitch "white" type of muscle to that of a slow twitch "red" muscle. Effects of stimulation program have been studied for stimulus periods ranging from 15 minutes per day to 24 hours per day for a total period of 28 days. As the exercise period is increased, beyond control, the oxidative enzyme activity shows the most change, from a slight increase at 15 min/day to an intense increase at 24 hrs/day. The least responsive enzyme is myofibrillar ATPase which shows slight changes at 8 hrs/day to a definite change at 24 hrs/day. The vascular bed also shows an increased density with exercise. The twitch times show a continuous slowing with all exercise programs; the most pronounced change occurring at 24 hrs/day. The net result of the electrically induced exercise program is a muscle which resists fatigue or sustains a given force for a longer period of time than the unstimulated control muscle. The results of these studies are important for applications of electrically induced muscle contraction where sustained muscle forces are required (paralyzed muscle and scoliosis correction). Further, these results support the hypothesis that muscle fiber conversion, (from fast twitch-glycolytic → fast twitch-oxidative → slow twitch-oxidative) results with increased usage of the muscle fiber.
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786 CHARACTERISTICS OF AFFERENT RESPONSES FROM HUMAN FINGER MUSCLES TO TENDON TAPS - A STUDY USING SURFACE ELECTRODES. K. S. Krishna Murthy and Philip L. Gildenberg. Div. Neurosurg. Univ. of Texas Med. School Houston, Texas 77030 U.S.A.

It is possible to record the responses of human finger muscle afferents to a phasic mechanical tap of the tendon using surface electrodes and employing signal averaging techniques. Experiments have been performed on conscious human volunteers. The recording electrodes (Beckman skin electrodes) were attached to the skin over the median or ulnar nerve at the wrist while a rubber pad was applied over the muscle tendon (first dorsal interosseus) at the metacarpo-phalangeal joint to cushion the taps made by a small rubber hammer. The signal was amplified using the preamplifiers of an electromyograph (TECA) and averaged using a Nicolet computer. The contributions from cutaneous afferents and tendon organs could be shown to be neglibible by employing Xylocaine to anesthetize the skin and muscle tendon. The major contributions to the recorded waveform appear to originate from muscle spindle receptors. The dispersion of afferent units (latency 5-15 milliseconds) is not severe enough to prevent qualitative observations being made. Maneuvers like removal of visual feedback or flexion of other limbs were found to influence the afferent response in a manner that could be explained on the basis of changes in fusimotor drive to the muscle under test produced by various maneuvers. Reflex efferent activity, when elicited, always occurred with a minimum latency of 20-25 milliseconds. Thus the afferent contributions could be easily distinguished from the averaged waveform.

787 DIFFERENCES IN TIMING OF MOVEMENT RELATED UNIT ACTIVITY IN MEDIAL AND LATERAL CAT PRECRUCIATE MOTOR CORTEX. E.J. Neafsey,* SPON. C. Sawyer, Mental Retardation Research Ctr., Dept. of Anatomy, Sch. Med., UCLA, Los Angeles, 90024.

Seventy-six movement related single units were recorded extracellularly from the pericruciate motor cortex (area 4) of 4 cats during bar pressing. Units were classified as PT (14) or non-PT (62) cells on the basis of their responses to pyramidal tract stimulation. EMG activity recorded from the brachialis, triceps, trapezius, and paraspinal muscles changed from the resting level 150-250 msec before the actual lifting of the bar began. This lift or elbow flexion phase of the movement was used as a trigger for averaging the unit activity for 2 sec before and 0.5 sec after its occurrence. The distribution of onset times of the movement related changes in neural activity was compared with the medial to lateral distribution of electrode penetrations into the pericruciate cortex. In each cat the units showing the earliest onsets of movement related changes in activity were recorded from the more medial penetrations into the precruciate cortex. Eighteen of 50 units (36%) recorded immediately rostral to the cruciate sulcus demonstrated changes in their firing rates more than 500 msec before the movement (early cells), while only 1 of 26 units recorded lateral to the end of the cruciate sulcus displayed such early changes. Three of the 6 PTN's in the precruciate group also displayed early changes in activity, while none of the 8 PTN's recorded lateral to the end of the sulcus showed these changes. The early units were located in the cortical region where electrical stimulation (Nieoullon, A. and Rispal-Padel, L. Brain Res. 105, 1976) produced shoulder movements, suggesting that they may be related to priming of neural pathways involved in the control of postural mechanisms preparatory to elbow flexion.

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788 VELOCITY OF INPUT AND OUTPUT OF MOTONEURONS. T. Pellmar and G. Somjen. Dept. of Physiol. & Pharm., Duke University, Durham, N.C. 27710

Muscle fibers innervated by fast conducting motor axons contract more rapidly than those innervated by slowly conducting axons (e.g. Wuerker, et al., J. Neurophysiol. 28: 85, 1965). It may by expected that faster motor units are accessible to commands from supraspinal sources more quickly than slower motor units. We have tested this hypothesis in motor units of the hindlimb of cats under chloralose anesthesia. The antidromic conduction velocity of motoneurons and the latency of intracellularly recorded post-synaptic potentials (PSPs) evoked by stimulation of the contralateral medullary pyramid (PT) and of the contralateral red nucleus were measured. A "pathway velocity" was computed by dividing the pathway distance by the latency of the PSP. Since the latency consisted of two components (axonal conduction plus interneuronal transmission), this pathway velocity was slower than the conduction velocity of the fastest descending axons addressed to the motoneuron. Pathway velocities of rubrospinal PSPs ranged from 50 to 130 m/sec. PT pathway velocities fell between 15 and 75 m/sec indicating that nearly all motoneurons receive, through spinal interneurons, input from "fast" PT fibers. Three-way comparison of motor axon conduction velocity, PT, and rubrospinal pathway velocities revealed no correlation. We conclude that, in the hindlimb muscles of cats, the speed of motor axon conduction and hence of motor unit contraction time is not related to the speed with which the motor unit can be activated by way of corticospinal and rubrospinal systems. (Supported by PHS grants NS 11933 and GM 00929.)

789 SOMATIC AND VISUAL INPUT TO VA AND VL THALAMUS OF MONKEY IN THE CONTEXT OF A CENTRAL MOTOR PROGRAM. Douglas D. Rasmusson*, Jane M. Macpherson* and John T. Murphy. Dept. Physiol., Univ.of Toronto, Toronto,Ont.,Canada.

Monkeys (Macaca speciosa) were trained to track an oscilloscope beam with flexion - extension movements about the wrist. On some trials they had to reposition the handle on the basis of visual feedback. On other trials a torque perturbation was applied to the manipulandum and the monkey had to reposition it using somatic feedback, either with or without corresponding visual information.

Following training, single unit recordings in the contralateral ventro-anterior and ventrolateral (VA/VL) thalamic nuclei were made while the monkey was performing this task. An initial sample of 64 cells has been found which discharge in relation to some aspect of the task. The somatic feedback channel clearly dominates as 63 of 64 cells responded to the torque perturbation. Many of these cells (17 of 63) showed patterns of firing which would be expected if they were related to single forearm muscles, e.g. excited by torque in one direction but inhibited in the opposite direction.

Convergence of somatic and visual feedback onto VA/VL cells during the task was common, with 43 of the 64 cells responding to both types of sensory disturbance. Passive stimulation of skin, muscles or joints revealed peripheral receptive fields in only 9 of the 64 cells and in all cases the linkage between the stimulation and cell firing was weak and variable. This suggests that there is an absence of somatic feedback to the VA/VL in the absence of a central motor program. When a central program is operative, however, cells in VA/VL thalamic nuclei are responsive to both visual and, especially, somatic feedback.

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790 PHYSIOLOGICAL PROPERTIES OF SPINDLES IN DORSAL MUSCLES OF THE CAT NECK. F.J.R. Richmond* and V.C. Abrahams. Dept. of Physiology, Queen's Univ., Kingston, Ontario, Canada K7L 3N6.

Physiological characteristics of spindles located in 3 dorsal neck muscles, splenius, biventer cervicis and complexus were examined by recording from single afferent fibres in upper cervical roots. Patterns of activity in response to stretches of controlled velocity and duration, to muscle contraction and vibration, and to succinylcholine were examined and correlated with axonal conduction velocities. In general, responses resemble those of spindles in the hindlimb, but there are some important differences. Conduction velocities were lower than those found in hindlimb so that spindle afferents with primary characteristics had axons with conduction velocities in the Gp II range. Spindles also demonstrated a wide range of sensitivities to length changes. This range of sensitivities may reflect differences either in the spindles themselves or in the mechanical properties of the muscle. The mechanical properties of the muscle do influence the pattern of spindle response and a proportion of spindles ceased to fire during passive muscle stretch due to off-loading of the part of the muscle containing the spindle.

The small perivertebral muscles have been found histologically to contain many small spindles. These spindles are extremely sensitive and may be strongly activated by even a small movement of overlying musculature. Such muscle receptors may be the source of afferent activity hitherto assigned to neck joint receptors.

* Supported by MRC of Canada.

791 CORRELATION OF BRAINSTEM AND SPINAL CORD ACTIVITY DURING HARMALINE-INDUCED TREMOR. J.F. Rowlands*, R. Llinás and A. Berthoz* (SPON: A. Pellionisz). Division of Neurobiology, Univ. of Iowa, Iowa City, Ia. 52242 and Lab. de Physiol. du Travail, 41, Rue Gay-Lussac, Paris, France.

The direct effect of the drug harmaline upon the inferior olive (IO) nucleus is now thought to be the cause of harmaline tremor. Simultaneous recordings from cat IO, spinal pathways, motoneurons and ventral roots (VR) were used to demonstrate the relation between the induced brainstem activity and the rhythmic muscular activation which is its assumed result. Cases are presented showing a very high correlation between rhythmic field potentials (FP) in the IO and spike bursts in contralateral and ipsilateral VRs. Correlated descending spinal volleys and motoneuron EPSPs follow IO FPs at latencies consistent with the hypothesis that IO firing causes activation of spinal motoneurons. Thus, we support the important ultimate effect of IO firing upon motoneurons during harmaline tremor. However, a more complex picture of the complete mechanism involved in harmaline tremor is suggested by an unexpected finding. In some instances of harmaline-induced activity, VR spike bursts consistently precede IO FPs even when activity in the IO and VRs is highly correlated. Analysis of these data suggests that they may represent a case in which inputs to the IO, possibly from the spinal cord, become significant in affecting its rhythmic response under harmaline. From preliminary experiments it seems that the IO may have multiple sites of harmaline-induced firing. It is envisioned that such organization may also help explain the fact that activity recorded in the IO can both precede and follow VR firing. Supported by PHS Grants NS-09916 and NS-05748 from NINCDS.

792 RELATION BETWEEN NEURAL ACTIVITY OF MESENCEPHALIC AND PONTINE RETICULAR FORMATION AND CONDITIONED LATERALIZED HEAD MOVEMENT IN RABBITS. Jerome N. Sanes* and J. S. Schwartzbaum* (SPON: D. W. McAdam). Psych. Dept., Univ. Rochester, Rochester, N. Y., 14627.

Electrophysiological research has implicated various regions of the brain-stem reticular formation (RF) in motoric functions. This study examined multi-unit and pauci-unit activity in mesencephalic and pontine nuclei of RF in relation to onset and laterality of conditioned head movements. Semi-restrained rabbits were trained to rotate their head left or right toward a lateralized visual stimulus for intraoral injection of rewarding fluid. Movements were detected through electromyographic (EMG) recording from neck musculature. Changes in neural activity (in the absence of sensory evoked responses) generally accompanied the earliest EMG indications of behavioral response and sometimes preceeded the latter by about 20 msec; rabbits typically required 100-120 msec after stimulus onset to initiate lateralized head movement. Placements in mesencephalic RF generally showed strong phasic contralateral relations to movement, i.e. higher activity during initiation and execution of contralateral than of ipsilateral head rotation. By contrast, placements in the pontine region (n. reticularis pontis oralis and pontis caudalis) generally displayed strong ipsilateral relations to movement. All neural responses diminished markedly in the absence of behavioral response to the stimuli. These laterality findings which can be partially understood in terms of afferent--efferent relationships of different RF regions, underscore the close link between activity of various RF structures and movement patterns.

793 SOME CENTRAL INFLUENCES ON ALPHA AND GAMMA TRIGEMINAL (V) MOTONEURONES. B.J. Sessle and L.F. Greenwood, Fac. Dent., Univ. Toronto, Canada M5G 1G6

Recently we described the effects of various peripheral stimuli on V motoneurones innervating jaw-opening (JO) or jaw-closing (JC) muscles (Greenwood and Sessle, In Mastication and Swallowing, Univ. Toronto Press, 1976). We have also studied the effects on JO and JC reflexes and single V motoneurones of bipolar stimulation (0.1-5.0 mA, 0.1 msec) of sensorimotor and orbital cortex (CX), lateral amygdala (AM) and cerebellum (CB). The effect of reversible cold block of synaptic transmission in V nucleus caudalis, which plays an essential role in V nociception, was also tested. In anaesthetized or decerebrate cats, alpha and gamma JC motoneurones were distinguished by the alpha's faster conduction velocity and lower threshold for antidromic excitation, its monosynaptic excitation from the V mesencephalic nucleus (site of muscle afferent cell bodies), and its lack of spontaneous firing with the jaw closed. No evidence for JO gammas was found. Compared with CX stimulation, AM and especially CB stimuli were relatively ineffective in modulating JO and JC reflex and motoneurone activity. The predominant effect of CX stimulation on JC reflex and alpha and gamma excitability was depression whereas excitation, or excitation followed by inhibition, was characteristic of CX regulation of JO reflex and motoneurone activity. The late inhibition of JO activity from somatosensory CX appeared to have a somatotopic dependency in that the most effective CX locus was the area receiving sensory input from the same peripheral site evoking the JO reflex. Another central region regulating JO activity was caudalis since caudalis block could reversibly depress JO reflex and motoneurone activity; JC reflex and alpha and gamma activity in contrast was unaffected. These findings provide further insight into the central neural organization underlying the initiation and control of jaw movements. (Supported by Canadian M.R.C.)

794 THE ORIGIN OF THE RUBROSPINAL TRACT IN PRIMATES AS SHOWN BY THE RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. Allan M. Smith and Jacques Courville
Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec, Canada.

Horseradish peroxidase was injected into either the cervical (C7 & C8) or lumbar (L3 & L4) enlargement of the spinal cord of two monkeys. Five injections of 0.4 µl of 30% HRP were made unilaterally into the grey matter of the spinal cord. Following a survival period of three days the animals were treated by histochemical procedures to reveal the enzyme and then stained with cresyl violet. All HRP labeled neurons projecting to the cervical and lumbar enlargements were located in the contralateral magnocellular red nucleus. No labeled neurons were found in the more rostrally situated parvicellular portion, nor were any labeled cells found in either rubral subdivision on the side ipsilateral to the injection. Evidence for a somatotopic arrangement of rubrospinal neurons within the magnocellular division was found. Cells projecting to the cervical enlargement are located medially whereas those projecting to the lumbar enlargement are located laterally in the magnocellular region. From these results the existence of a contribution to the rubrospinal tract from the parvicellular portion of the red nucleus seems unlikely.

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795 LONG-TERM RECORDING OF ELECTRICAL ACTIVITY OF MAMMALIAN NERVES FOLLOWING AXOTOMY. R.B. Stein, J. Jhamandas* and T.R. Nichols. Dept. Physiol., Univ. Alberta, Edmonton, Canada T6G 2H7

Recently, methods were described for recording for long periods from intact nerves of the cat using silastic cuffs in which three or more circumferential electrodes have been inserted (Stein *et al.*, Can. J. Neurol. Sci. 2: 235, 1975). These methods have now been extended to record from severed nerves which were placed in a cuff whose distal end has been sealed. Changes in the state of the axons were monitored by recording the impedance of the nerve, the compound action potentials and voluntary activity at various distances from the sealed end (see also DeLuca and Gilmore, Science 191: 193, 1976). The following sequential changes were observed following axotomy: 1) the impedance, the compound action potentials and the neural conduction velocity all increased for a couple of days after cutting the nerve, presumably due to swelling of the cut end, 2) the three parameters indicated in 1) above all declined for a week or so, presumably due to degeneration back from the cut end, 3) the three measures all increased for about a week due to attempted regeneration of the nerve through the sealed end, 4) the compound action potentials declined slowly with a time constant of 1-2 months. The conduction velocity declined steadily, but even more slowly. This was interpreted to represent a slow, continuous decline in fiber diameter of axons which could not find an end organ. This decline was more rapid in tight-fitting cuffs, perhaps representing a selective effect of compression on the largest fibers. At the same time, impedance increased for a month or two due to ingrowth of connective tissue. Voluntary activity during a stereotyped task (walking on a treadmill) declined more rapidly than the compound action potentials and was difficult to observe more than a month after axotomy. The implications of these results for the neural control of artificial limbs will be discussed.

796 POSTURAL ANKLE TREMOR. Robert N. Stiles and Robert R. Rietz*. Dept. of Physiol. and Biophysics, Univ. Tenn. Cntr. Hlth. Sci., Memphis, TN, 38163.

Spectral analysis was performed on ankle tremor records from five normal subjects. Each seated subject supported one leg on the ball of the foot with the heel elevated 2-5 cm. Ankle tremor was detected with an accelerometer mounted on the dorsal aspect of the knee. During each experiment, the subject held the heel elevated without rest for 10-45 min. Bipolar, surface EMG recordings were obtained from the soleus muscle. Results included a negative relation between the root-mean-square (rms) displacement amplitude and frequency for these tremors. In general, this tremor occurred at different steady-state frequencies (each calculated over 16 sec), decreasing from 7-8 Hz to 5-6 Hz with an increase in rms displacement of 100-1,000 times. The relation between the log values of frequency and displacement amplitude was very similar to that found previously for hand tremor. Also, the large-displacement tremors obtained with prolonged elevation of the heel had frequency and displacement values similar to those for clonus-like oscillations. These clonus-like oscillations occurred in normal subjects after repeated induction of damped, or die-away, oscillations of the ankle. A possible role for neural feedback factors was indicated by an increasing rms amplitude of the demodulated soleus EMG (calculated at the tremor frequency) with increasing displacement amplitude of these tremors. This value of the EMG increased over a range of about 4 to 40 μV as the rms tremor displacement increased from about 4 to 4,000 μm. Also, the coherence between the demodulated EMG and the ankle tremor was about 0.7 for tremor records having rms displacement > about 30 μm. These results suggest that the frequency and amplitude of postural ankle tremor may be determined by a mechanism similar to that for postural hand tremor, i.e., a mechanical-reflex oscillator mechanism. (Supported in part by USPHS Grant NS-08692).

797 AN AUTORADIOGRAPHIC STUDY OF THE RUBROOLIVARY TRACT IN THE RHESUS MONKEY. Norman L. Strominger, Timothy C. Truscott, Richard A. Miller* and G. James Royce. Department of Anatomy, Albany Medical College, Albany, New York 12208.

Tritiated leucine (1 μL; 20 μCi/μL) was injected unilaterally into the rostral part of the red nucleus of 10 rhesus monkeys. Animals were killed after 5-40 day survivals. Previous studies with this species have demonstrated that the rostral red nucleus, comprised of perikarya with finely granular Nissl material, gives rise to fibers which from the rubroolivary tract (Miller and Strominger, JCN 152:327, 1973).

Study of sections prepared by autoradiographic techniques and stained with azure-B or cresyl violet showed that the rostral part of the red nucleus emits axons which enter the central tegmental tract and descend entirely uncrossed as far as the inferior olivary complex. Silver grains were present in the pedunculopontine nucleus located between the dorsolateral margin of the superior cerebellar peduncle and the inferior colliculus, in the nuclei reticularis pontis oralis and pontis caudalis and in the nucleus reticularis gigantocellularis. Injections restricted to different regions of the red nucleus elicited patterns of silver grains differentially distributed within the principal inferior olivary nucleus. However, the precise topographic organization of this pathway could not be determined because of the size of the injections. Grains, in some cases copious in amount, sometimes were seen in the ipsilateral medial accessory olivary nucleus but never in the dorsal accessory nucleus. No evidence was found suggesting the presence of projections to the cerebellum from the rostral part of the red nucleus. (Supported by NIH grant NS-12208.)

798 COMPARISON OF HUMAN MUSCLE SPINDLE ACTIVITY DURING VOLUNTARY CONTRACTION OF THE MUSCLE OF RECEPTOR ORIGIN AND A REMOTE MUSCLE. Alfred J. Szumski and Doris Burg*. Dept. Physiol., Med.Coll.Virginia, Richmond, VA. 23298

Muscle spindles were isolated, identified and recorded in the Median n. of unanesthetized human subjects, using tungsten semimicroelectrodes (110-115 KOhm). Single receptors were isolated in the nerve by minute manual manipulations of the electrode; the receptor was identified by criteria which included passive stretch and response during the electrically induced twitch and the phasic reflex; the muscle of receptor origin was identified by electrical stimulation through the recording electrode located at the muscle nerve fascicle. Muscle activity was monitored electromyographically and with a force displacement transducer. Single spindle afferents from a resting wrist or hand muscle recorded in the Median n. repeatedly increased their discharge frequency during a restricted voluntary contraction of the remote Quadriceps muscle. Spindles which were spontaneously active were facilitated most readily; the facilitation occurred with a nearly synchronous onset of the remote extrafusal contraction, and usually showed a pronounced after-effect. The majority of spindle afferents recorded during a voluntary contraction of the muscle of their origin increased their discharge frequency in spite of muscle shortening. Also, using the electrically induced twitch as a test of spindle dynamic innervation, spindles with a low dynamic sensitivity were, after a short latency, coactivated during the voluntary muscle contraction; spindles with a high dynamic sensitivity decreased firing immediately at the onset of the contraction and fired in a burst-like manner during relaxation. The results support the suggestion that a voluntary motor act can facilitate spindles in a resting muscle. In contrast, the spindle discharge pattern in a muscle during an isometric voluntary contraction depends on the specific receptor innervation.

799 EFFECTS OF ACUTE BRAIN INJURY ON THE EXCITABILITY OF NEURONES IN THE CAT MOTOR CORTEX. Floyd J. Thompson, Dept. of Neuroscience, Coll. Med., Univ. Fla., Gainesville 32610.

It has been suggested that cortical injury is accompanied by a cortical shock which results in an inactivation of cortical neurones adjacent to the site of injury. It would therefore be of considerable interest to understand more completely the nature of the effects on surviving cortical neurones adjacent to a site of brain injury.

The following experiments were conducted to examine the effects of acute cortical injury on the electrical excitability of cortical neurones in the motor cortex of the cat. The pericruciate motor cortex was exposed by craniotomy and covered with a closed chamber. Bipolar electrodes were inserted into 8 hindlimb muscles and 8 forelimb muscles to record EMGs. Electrical thresholds for eliciting EMGs by cortical stimulation were examined at multiple positions on the motor cortex. An acute cortical injury of a portion of the examined cortex was produced by surgery, suction, or radio frequency methods. The elctrical thresholds for eliciting EMGs by cortical stimulation were re-examined and compared to the pre-lesion control values.

These experiments have shown that the thresholds for the cortically elicited EMGs were not increased significantly by the adjacent cortical injury. These data suggest that the electrical excitability of cortical neurones is not depressed as a result of brain injury in a contiguous region of the motor cortex.

800 A MOTONEURONE WITH TWO SPIKE INITIATION SITES LOCATED IN DIFFERENT GANGLIA OF THE STOMATOGASTRIC NERVOUS SYSTEM OF THE ROCK LOBSTER. Jean-Pierre Vedel[+] and Maurice Moulins[+] (SPON : A.I. Selverston). CNRS, INP.10, 13274 Marseille, France.

The stomatogastric nervous system of the rock lobster includes two ganglia (oesophageal OG, stomatogastric STG) which are connected by the stomatogastric nerve (stgn). OG and STG neurons organize four neural networks assuming the motor programs of the four successive parts of the foregut : ie. oesophagus - cardiac sac - gastric mill - pylorus. The cell body of one motoneurone innervating the cardiac sac muscle is located in the STG. All its axonal branches have a motor function excepted one which projects directly to the OG. Two kinds of spontaneous activities caracterize this neuron :

- burst discharges initiated at the OG level and propagated towards the STG in the stomatogastric nerve,
- tonic discharge initiated at the STG level which is propagated towards the OG. Both discharges can cause muscle contraction. This neurone exibits bidirectional spike propagation (antidromic-orthdromic) in the stg nerve which has been confirmed by collision experiments.

The evidence of spike initiation sites in different ganglia supports the possibility of a motoneuronal activity involved in different motor programs. Moreover the occurence of two spike initiating regions functionnally may be considered as a reciprocal control system over motor patterns initiated in the two separated ganglia.

801 COORDINATION OF TREADMILL GALLOPING BY CATS. Mary C. Wetzel, Kent S. Norgren*, Barbara L. Eisenstein* and Rex C. Anderson*. Dept. Psychol., Univ. Ariz., Tucson, Ariz. 85721

Cats galloped on a motor-driven treadmill either to avoid aversive stimulation (noisy air jet or shock) or for food reward alone. Kinematic data were taken for 5-10 successive strides at a constant velocity. Steady-state galloping (an invariant footfall order) was seen at velocities from 2.3-6.1 m/sec and classified as rotatory, transverse, or half bound by conventional criteria. Mixed galloping was also seen, in which the footfall order converted (between 2 successive strides) from one to another of the 3 galloping patterns. Major findings were that: 1) Galloping tended to be slower for food reinforcement than when aversive stimuli were used. 2) Any steady-state pattern could be used at any velocity,but cats working for food tended to use the rotatory gallop. 3) Stance (down) durations were relatively invariant, even in mixed galloping. 4) Overall, the tightest (but not obligatory) hindlimb-to-forelimb timing was between trailing limbs, which could be on opposite sides of the body, to argue against a pre-eminently ipsilateral propriospinal influence. 5) In mixed galloping the stride shifted as a unit, almost entirely because swing (up) duration could vary from 150-300 msec. Contrary to previous reports, it was concluded that the swing has flexible neural control. Perhaps versatile galloping repertoires help avoid fatigue in individual limbs. (Supported by USPHS grant NS 11491)

802 BIOMECHANICS OF THE JUMPING CAT. Michael R. Zomlefer, Felix E. Zajac and William S. Levine. Dept. of Elec. Eng., Univ. of Maryland, College Park, Md. 20742

Cats were trained to jump to their maximal vertical height from a force platform. The "crouch" to "lift-off" phase of the jump begins with a period of about 75 msec in which the animal's entire foot remains in contact with the ground, while he moves his center of gravity forward from a position over the heel to a point directly over the toes. During this interval, stroboscopic techniques showed that the knee angle remains constant at about 48° while the hip angle extends slightly from 94° to 125°. The ankle angle decreases from an initial value of 78° to less than 60°. In the next 75 msec, the heel leaves the ground (heel-off), and the center of gravity is then driven upward as the ankle, knee, and hip angles extend to angles of 125°, 150° and 160°, respectively. Extensors VL and MG are both active during crouch to lift-off, at which time they abruptly become inactive to indicate that while VL was isometric before heel-off, MG underwent an active lengthening contraction. Flexor TA was active before lift-off to provide stability and afterwards to brake the ankle joint. Using a simple dynamic model for the jumping cat, the resultant torque about the ankle joint was estimated to reach 4.4 N-m, which corresponds to a minimum ankle extensor force of 245 newtons. Peak forces generated by the ankle extensors were thus shown to be at least 54% of their maximum tetanic force. (supported by NIH grants NS 11971 and NS 11518, a NDEA fellowship to M. R. Z., and Biomedical Sciences Support to F. E. Z.)

Narcotics and Drugs of Abuse

803 HYPERTHERMIC RESPONSES TO CENTRAL AND PERIPHERAL INJECTIONS OF MORPHINE SULFATE IN THE CAT. Wesley G. Clark and H. Rick Cumby*. Dept. Pharmacol., Univ. of Texas Health Science Center at Dallas, Dallas, TX 75235.

Although it is well known that morphine causes hyperthermia in the cat, the mechanisms responsible are still poorly understood. In this initial study, injections into adult cats were made via indwelling lateral (lv) or third (3v) ventricular cannulae or jugular venous catheters. Body temperature (T_{rp}) was recorded automatically from a thermocouple implanted into the retroperitoneal space. Ambient temperature (T_a) was 22 ± 1°C unless otherwise indicated. All experiments were crossover in design with each cat allocated various doses of morphine sulfate (MS), saline, etc. in random order. Cats (novices) which had not previously received MS (lv, 3v) or had not received MS for at least 11 days (iv) were given a dose of MS within the ranges indicated in the table below, which also indicates the mean increase in T_{rp} produced by the lowest and highest doses and, in parentheses, the number of cats given each dose. The maximum mean rise in T_{rp} during comparable time periods after saline injections was 0.3°C. Seven cats were given intraventricular injections of a given dose of MS (20-50 µg) repeatedly at intervals ranging from one to four days to determine an interval at which tolerance development was minimal. An interval of three days appeared satisfactory. In more extensive crossover studies, cats were then given a series of doses of MS at intervals of three or more days. Comparison of the results, listed partially in the table below, (1) confirms that a three-day interval between injections avoids significant tolerance as indicated by the similarity of the responses of the novice cats to the responses in the crossover experiments and indicates (2) that 3v injection is as effective as lv injection for a given concentration of MS and (3) that MS is at least 500 times more potent when given centrally than when given iv. In cats given the larger doses, the responses usually

Route (volume)	LATERAL VENT. (0.10 ml)	THIRD VENT. (0.05 ml)	IV (0.10 ml/kg)
Dose range	10.0-50.0 µg	2.5-20.0 µg	1.0-10.0 mg/kg
A. ΔT (novice)	1.4-2.3°C (2-4)	0.3-2.0°C (3)	0.8-2.7°C (3)
B. ΔT (crossover)		0.6-1.9°C (8)	0.6-2.5°C (6)

began with shivering within 15 min and reached maximum within 2-3 hr. Injections of still larger doses (40-1250 µg) into the 3v produced maximal hyperthermias of about 3°C. In the remaining experiments MS (10-20 µg) was injected into the 3v. Six cats were given MS (10 µg) or saline at each of three T_as in a Latin-square design arranged so that the interval between MS injections was at least five days. The table below lists the mean increases in T_{rp} with the range in parentheses. The increments in

T_a	34°C	22°C	4°C
MORPHINE	1.9°C (1.3-2.5)	1.6°C (1.0-2.3)	1.5°C (0.9-2.2)
SALINE	0.7°C (0.5-1.3)	0.2°C (0.0-0.9)	0.1°C (0.0-0.3)
Δ	1.2°C	1.4°C	1.4°C

T_{rp} were similar regardless of T_a. This suggests that MS elevates the thermoregulatory set-point or stimulates a pathway between cold sensors and heat conservation and production effectors. Possibilities that histamine, prostaglandins and/or cyclic AMP might be required for the hyperthermic response to MS were investigated by giving various agents during the response. Central injection of the histamine H_2-receptor antagonist metiamide (1 mg), which blocks a hyperthermic response to histamine, did not alter the response to MS. Large doses of indomethacin (2mg/kg, iv) were likewise ineffective so that neither pyrogenic contamination nor prostaglandins seem to be involved. 3-Isobutyl-1-methyl xanthine (400µg, 3v), a phosphodiesterase inhibitor, also produced no change so that cyclic AMP is not likely to play a role in MS-induced hyperthermia. Supported by NIH Research Grant NS 08618.

804 METHADONE DURING PREGNANCY: ASSESSMENT OF BEHAVIORAL EFFECTS IN THE RAT OFFSPRING. Donald E. Hutchings*,Howard F. Hunt*, James P. Towey*, and Howard S. Gorinson * (SPON: Michael Potegal) Psychiatric Institute, 722 West 168th Street, New York, N.Y. 10032

Methadone produces a neonatal withdrawal syndrome which has been described as more severe, prolonged and difficult to control chemotherapeutically than heroin. Moreover, many methadone infants show symptoms of excessive sucking, voracious appetite, hypertonicity, and disturbed sleep that may persist for several weeks after discharge, and hyperactivity that lasts up to two years of age. Followup studies suggest that both methadone and heroin produce a syndrome of minimal brain dysfunction consisting of hyperactivity, extreme mood lability, and impaired fine motor coordination. To study the possible long-term behavioral effects, rats were administered methadone hydrochloride by gastric intubation. Beginning on Day 8 of pregnancy, a drug group received 5 mg/kg and after 4 days was increased to a daily maintenance dose of 10 mg/kg, with the final dose given on Day 22. This treatment regimen produces maternal and fetal blood levels equivalent to daily human maintenance doses of approximately 40-80 mg of methadone. An intubation control group received sterile water alone on the same gestation days and a non-treated control group was left undisturbed during pregnancy. All experimental and control litters were fostered at birth to untreated mothers. Behavioral analysis of the methadone offspring revealed a substantial reduction in diurnal activity at weaning. On an operant conditioning task, methadone offspring exhibited rates of response that greatly exceeded the controls but there was no evidence of a learning deficit. Independent assessment of activity indicated that the high rate of response seen among the methadone treated offspring was associated with a high level of generalized activity. The behavioral effects observed were seen only in male offspring; evidently the females were protected in utero from methadone effects reflected in behavior. Interestingly, these observations, particularly with respect to the sex-dependent hyperactivity, parallel the clinical observations of chronic hyperkinesis seen largely among male offspring of both heroin and methadone addicted mothers.

805 COMPARISON OF MORPHINE-INDUCED AND STIMULATION-PRODUCED ANALGESIA AT COINCIDENT PERIAQUEDUCTAL CENTRAL GRAY (PAG) LOCI OF THE RAT. V.A. Lewis* and G.F. Gebhart. Dept. Pharmacol., Sch. Med., U. of Iowa, Iowa City, IA. 52242.

The effects of intracranially administered morphine (MOR; 17.5 nmole/0.5 µl) and focal brain stimulation (FBS; 120 µA, 60 Hz, 1 msec duration bipolar pulse pairs) were compared at coincident PAG loci employing a 3 x 3 factorial design. Eighty male Sprague-Dawley derived rats were surgically implanted with chronic indwelling cannula-electrode units (30 gauge guide cannula, 35 gauge injection cannula). MOR and FBS were evaluated in three analgesiometric tests: the hot plate test (HP), the tail flick test (TF), and a pinch-squeal test (PS). Comparison between MOR, FBS and artificial cerebrospinal fluid control (CSF) were made on two test days in animals assigned to one of nine experimental groups. On day 1, animals were treated in sets of three groups, each set receiving either MOR, FBS or CSF. On day 2, the three groups from each day 1 set received either MOR, FBS or CSF thus creating 9 uniquely treated experimental groups.

Analysis of the day 1 data revealed that both MOR and FBS significantly prolonged response latencies in the HP and TF tests, the TF test being more sensitive to the treatments than the HP test. Only MOR significantly altered responding in the PS test. Following the day 2 treatments, it became apparent that previous CSF treatment on day 1 enhanced MOR's effect on day 2. In the HP test, a significant decrease in MOR's effect was seen on day 2 when preceded by MOR on day 1. Data suggesting FBS-FBS tolerance was also apparent on day 2 in all three tests. Histological evaluation of cannula-electrode placements revealed that marked analgesia after FBS resulted when the electrode was in the ventrolateral PAG whereas marked analgesia subsequent to MOR injection resulted when the cannula was medial in the PAG and near the aqueduct. Ink which was injected via the injection cannula prior to animal sacrifice was found in the aqueduct and 4th ventricle (as well as the PAG) in the majority of animals exhibiting marked analgesia after MOR injection during the experiment.

These data provide no evidence that the more efficacious FBS analgesic PAG loci are congruent with the more efficacious MOR analgesic PAG loci. (Supported by USPHS grants NS 12114 and GM 22026.)

806 A POTENT, LONG-LASTING ENKEPHALIN ANALOG WITH MORPHINE-LIKE ANALGESIC PROPERTIES. Agu Pert, Candace Pert and Kathryn Robison*. Section on Biochemistry, Adult Psychiatry Branch, NIMH, Bethesda, MD. 20014. Bosco T.W. Fong* and Jaw-Keng Chang*. Bioproducts Dept., Beckman Instruments, Palo Alto, CA.

Met- and Leu-enkephalin, two pentapeptides isolated originally from pig brain (Hughes et al. Nature, 258, 577) and later from calf brain (Simantov and Snyder, Life Sci, 18, 781), possess potent morphine-like pharmacological activity in the guinea pig ileum and mouse vas deferens. In the brain, however, met-enkephalin requires quantities forty-fold higher (120µg) than morphine to elicit a mild, transient analgesia which is no longer detectable 3-4 min after microinjection into rat periaqueductal grey matter. Leu-enkephalin, by contrast, does not significantly increase the latency of the "tail-flick" response.

Although met-enkephalin binds almost as tightly as morphine to opiate receptors in rat brain homogenates, its receptor affinity is rapidly and completely destroyed by enzymatic peptidases found therein (Chang, Fong, Pert & Pert, Life Sci, in press). Thus, rational design of an active enkephalin analgesic depends upon resistence to enzymatic degradation rather than increased affinity for opiate receptors. BI 274, a close analog of enkephalin, was found to completely resist enzymatic inactivation even after incubation for 2 hours at 37°C with a concentrated rat brain homogenate (20 volumes). Its affinity for opiate receptors in rat brain homogenate is 4×10^{-7}M, about 1/4 as potent as morphine and half as potent as met enkephalin. Intracerebral microinjection of only 10µg of BI 274 results in a morphine-like analgesia (increase in tail-flick latency from 4.5 to 11.3 seconds) which is reversible by naloxone and persists for 1-2 hours. Intravenous injection (50mg/kg), however, produces nc significant effect, presumably because BI 274 fails to penetrate the blood-brain barrier.

A long-lasting peptide of the enkephalin class is a useful tool for evaluating the ability of this class to produce tolerance and physical dependence. After daily intracerebral microinjections of morphine (15µg), tolerance to its analgetic effect appears on day 5 and by day 7 this dose no longer elicits significant analgesia. On day 8, 15µg of BI 274, which initially produced a maximal analgetic response, failed to elicit analgesia in morphine-injected, but not saline-injected control rats. Thus, analgetic cross-tolerance between morphine and an opiate peptide was demonstrated. Intestinal strips from guinea pigs which have been chronically treated with morphine, are also less responsive to enkephalin (Kosterlitz et al. Nature, 260, 602). It is conceivable that an opiate peptide so similar to a natural brain component will be less able to activate mechanisms which result in tolerance and physical dependence. The effects of chronic microinjection and ventricular perfusion of BI 274 are thus being carefully examined.

807 INCREASE IN OPIATE LIKE FACTORS UPON ANALGETIC ELECTRICAL STIMULATION IN RAT BRAIN. H. Akil*, S. Watson, and J. Barchas. Dept. Psychiatry, Stanford University, Stanford, CA. 94305.

Recently, a family of peptides with morphine-like properties has been isolated and identified (Hughes *et al.*, *Nature*, 1975). Opiate-like factors were also detected employing displacement of opiate-specific binding (Terenius & Whalstrom; Pasternak *et al.*, *Life Sci.*, 1975). Some of these peptides, the enkephalins, were shown to exhibit cross-tolerance with morphine (Waterfield *et al.*, *Nature*, 1976) and analgesia upon ventricular injection (Belluzzi *et al.*, *Nature*, 1976). However, little is known about their endogenous roles or their modulation by environmental events. Electrical stimulation of periaqueductal sites has been shown to produce potent analgesia (c.f. Liebeskind, Mayer & Akil, *Adv. Neurol.*, 1974). We, therefore, examined the effect of analgetic electrical stimulation in the periaqueductal grey on levels of brain opiate-like factors.

Twelve animals were chronically implanted with bipolar electrodes in the central grey. Changes in pain responsiveness (tail flick) were systematically examined, employing trains of bipolar square pulses (200 μsec, 20 Hz). In the screening phase, current levels were chosen to produce analgesia (doubling of tail flick latency), which could be continuously observed for 20 minutes. The experimental animals were then stimulated for 20 min and sacrificed immediately; the control group was not stimulated. Opiate-like factors were measured by inhibition of specific opiate binding of ^{3}H-naloxone to brain homogenates. The peptides were extracted following the procedure of Pasternak *et al.* (1975) and each sample assayed in triplicate. Analgetic stimulation induced a 35% increase in whole brain opiate-like factors in the experimental group ($p < .02$). It, therefore, appears that a correlation exists between pain responsiveness in the rat and levels of opiate-like factors in the brain.

808 EVIDENCE IN MONKEYS FOR THE PRODUCTION OF PHYSICAL DEPENDENCE BY COCAINE. H. L. Altshuler, M. N. Hubler*, D. W. Sanders* and N. R. Burch*. Texas Research Institute of Mental Sciences and Baylor College of Medicine, Texas Medical Center, Houston, Texas 77025.

Rhesus monkeys were treated chronically with low doses of cocaine HCl (3 mg/kg, tid, sc) for fifteen months to evaluate the degree to which chronic exposure to cocaine produces tolerance or physical dependence. The animals received cocaine HCl, 3 mg/kg, subcutaneously three times daily to provide chronic exposure to the drug. Electroencephalographic (EEG) and behavioral data were collected to provide quantitative indices of changes in response to acute intravenous challenge doses of the drug (3 mg/kg, IV) during the chronic phase of the study. Minimal changes in stereotypic behavioral responses to both acute and chronic doses were observed. The EEG data was analyzed by period analysis to determine descriptors of EEG responses to cocaine which occurred during each experiment and provide summary data for the entire study. The EEG response to acute IV doses of cocaine were complex, and those responses changed during the fifteen month study, with some aspects of the EEG response becoming attenuated and some becoming potentiated as the result of the chronic treatment. After abrupt cessation of the chronic cocaine dosage regimen, behavioral and EEG changes which resembled a type of abstinence syndrome were noted. These experiments suggest that cocaine produces a form of physical dependence analagous to, although in many ways different from the physical dependence produced by a number of drugs of abuse. (Supported, in part, by NIDA Grant No. 1R01DA00799)

809 AUTORADIOGRAPHIC LOCALIZATION OF OPIATE RECEPTORS IN SPINAL CORD AND LOWER MEDULLA OF THE RAT. Samir F. Atweh* and Michael J. Kuhar. Dept. Pharmacol., Johns Hopkins Univ. Sch. of Med., Baltimore, Md. 21205.

The localization of opiate receptors in the spinal cord and lower medulla has been elucidated by the autoradiographic identification of ^{3}H-diprenorphine (a potent opiate antagonist) binding sites. Layers I and II (marginal cell zone and substantia gelatinosa proper) of the spinal cord, and the substantia gelatinosa of the spinal trigeminal nucleus, had high densities of opiate receptors. These findings support the suggestions that opiates can affect nociceptive stimuli at the level of the spinal cord. High densities of opiate receptors were also found on various components of the vagal system in the lower medulla. These sites included the vagus nerve, nucleus tractus solitarius, nucleus commissuralis, nucleus intercalatus, parts of the nucleus ambiguus and nucleus originis dorsalis vagus. These receptors could provide some basis for the known side effects of opiates associated possibly with the vagal system. The area postrema also had a high density of opiate receptors indicating that the nausea caused by opiates could be due to direct action at this site. ^{3}H-etorphine (a potent opiate agonist) binding sites showed the same distribution as those for ^{3}H-diprenorphine. Processing the tissues by different procedures designed to prevent diffusion also resulted in the same distribution of binding sites. We conclude that, in these brain regions, opiate receptors are: 1) highly associated with areas receiving small, primary afferent fibers; 2) strategically placed to modulate noxious stimuli as well as possibly explain some visceral side effects of opiates.
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810 ENKEPHALIN: INTRAVENTRICULAR SELF-ADMINISTRATION IN THE RAT. James D. Belluzzi, C. David Wise* and Larry Stein. Wyeth Laboratories, Philadelphia, Pa. 19101.

Centrally administered enkephalin mimics morphine's capacity to induce analgesia in the intact rat (Belluzzi et al, Nature 260, 625, 1976). Here we report self-administration data which suggest that methionine -enkephalin also shares morphine's addictive or reinforcing properties. Naive rats with permanently-indwelling cannulas in the lateral ventricle were confined in a Skinner box for 2-4 days. Each lever press delivered 1 µl of fluid to the brain in 1 second. Different groups had access to methionine-enkephalin (10 µg/µl, pH 5.8), morphine sulfate (0.5 µg/µl, pH 5.8), or Ringer's solution (pH 5.8). For a fourth group of "yoked" controls, lever-press responses were recorded but had no other consequences; however, each yoked rat received methionine-enkephalin whenever its arbitrarily assigned partner in the enkephalin group responded. Sustained and sometimes avid self-administration was obtained with enkephalin and morphine, but not with Ringer's solution. One rat responded 871 times for enkephalin in the final 16 hours of testing, and a second rat died after 397 infusions in the initial 38 hours. In 6 out of 7 pairs, response rates of enkephalin rats in the final 12 hours exceeded that of their yoked partners [mean ratio (enkephalin/yoked) $\pm$ S.E.M. for 7 pairs = 5.04 $\pm$ 1.66]. This result rules out any actions of enkephalin unrelated to reinforcement as explanations of enkephalin self-administration; furthermore, failure of self-administration with pH 5.8 Ringer's solution indicates that enkephalin self-administration is not an artifact of acid pH. Acquisition curves for morphine were sharper than those for enkephalin, despite the 20-fold greater concentration of the peptide. We conclude that centrally-administered enkephalin exerts an opiate-like reinforcing action, although its potency may be only 1/20 or less of that of morphine.

811 SIGNAL DETECTION ANALYSIS OF CHANGES IN NOCICEPTION IN THE MONKEY FOLLOWING OPIATE ADMINISTRATION TO SPECIFIC BRAIN AREAS. C. Thomas Bennett, Robert C. Hulsebus* and Thomas E. Bevan.* Psychology Group, Biomedical Laboratory, Edgewood Arsenal, APG, MD 21010.

It has been shown that opiates will reduce perceived intensity and discriminability of two nociceptive stimuli in a Signal Decision Theory paradigm (Hulsebus, et al., Fed. Proc., 35: 386, 1976). However, it is not known whether opiates act in the same brain area to reduce both perceived intensity and discriminability. To answer this question, five rhesus monkeys were trained to discriminate two aversive electrocutaneous stimuli. In these animals, guide cannulae were then stereotaxically directed at brain areas known to have high concentrations of opiate receptors. Microinjections of morphine (50 µg/µl/site) were made bilaterally in, or near, the ventral thalamus, amygdala, or mesencephalic periaqueductal gray (PAG). Except in one case, no changes in nociception occurred following application of morphine to the amygdala (N = 6). In the ventral thalamus and PAG, a significant decrease in perceived intensity of nociceptive stimuli followed morphine injection (8 out of 9 cases). In 6 out of these cases, no change in discriminability was produced. It is suggested by this data that morphine acts in the ventral thalamus and PAG to reduce perceived intensity without affecting nociceptive discriminability.

812 FAILURE OF ENKEPHALIN TO STIMULATE DOPAMINE METABOLISM IN THE CORPUS STRIATUM OF THE RAT. S. Berney and O. Hornykiewicz. Clarke Institute of Psychiatry, 250 College Street, Toronto, Canada, M5T 1R8.

A recent report of Hughes et al (Nature 258: 577, 1975) identified enkephalin (H-TRY-GLY-GLY-PHE-MET-OH) as an endogenous pentapeptide with agonist opiate receptor activity. Experiments were performed to evaluate the ability of morphine or enkephalin to modify dopamine (DA) metabolism as measured by changes in the concentration of homovanillic acid (HVA) in the corpus striatum of the rat. The agents were injected into the anterior horn of the left lateral ventricle of conscious, free moving rats which were sacrificed 30 minutes later. Behavioural effects were observed. HVA was estimated in pooled (2) corpora striata ipsilateral to the side of injection of the agents and saline respectively. The administration of 5 ug of morphine significantly ($p<0.05$) increased HVA in the corpus striatum (0.96 ± 0.04 (5); ug/g $\pm$ S.E.M. (n)) compared to saline controls (0.73 ± 0.04 (7)). In contrast, 100 ug of enkephalin did not modify HVA in the corpus striatum (0.70 ± 0.02 (3)). Enkephalin, however, did produce some behavioural changes. Throughout the course of the experiment the rats receiving enkephalin displayed piloerection and a hunched back posture but no decrease in spontaneous locomotion. In contrast, morphine produced a depression of locomotor activity and analgesia, as assessed by tailpinch. In the dose tested, enkephalin was devoid of these characteristic opiate effects and failed to modify DA metabolism. In conclusion, intraventricular administration of 100 ug of enkephalin in the rat does not produce any of the pharmacological or biochemical effects characteristic for narcotic-like compounds.

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813 PARTICIPATION BY 5-HYDROXYTRYPTAMINE IN MORPHINE EFFECTS ON EVOKED RESPONSES IN HYPOTHALAMUS AND PINEAL BODY. **T.F. Burks and N. Dafny. Departments of Pharmacology and Neurobiology, The University of Texas Medical School at Houston, Houston, Texas 77030.**

Morphine effects on sensory (acoustic) evoked responses in unanesthetized rats were studied before and after the administration of drugs which alter 5-hydroxytryptamine (5-HT) neurotransmission in the central nervous system. Permanent electrodes (60μ in diameter) were implanted stereotaxically in the ventromedial hypothalamus (VHM) and pineal body (PB) in male Sprague-Dawley rats (200-300g). Animals were allowed to recover from surgery for one week before experiments were begun. Evoked responses were recorded in all animals before and after administration of morphine (10 mg/kg i.p.). Morphine in this dosage altered (either increased or decreased) amplitudes of the evoked responses in both structures in approximately one-half of the animals. Each animal was retested with morphine after treatment with a drug which interacts with CNS 5-HT. Treatment with tryptophan (25 mg/kg i.p.) increased the total percentage of animals which responded to morphine. Tryptophan also increased the percentage of animals in which response amplitudes were increased by morphine. After treatment with parachlorophenylalamine (300 mg/kg i.p. 24 hours previously), on the other hand, the predominant effect of morphine was to decrease response amplitudes in both VMH and PB. Similarly, treatment with cinanserin (15 mg/kg i.p.) tended to shift the effect of morphine towards decreases in response amplitudes. While there were differences between the two structures in the specific response components affected, alterations in 5-HT changed responses to morphine. The results suggest that 5-HT participates in the actions of morphine on sensory evoked responses in VHM and PB. (Supported by USPHS grant DA 00803.)

814 SEROTONINERGIC LINKS IN RESPONSES OF CAUDATE NUCLEUS AND SUBSTANTIA NIGRA TO MORPHINE. **N. Dafny and T.F. Burks, Departments of Neurobiology and Pharmacology, The University of Texas Medical School at Houston, Houston, Texas 77030.**

The effect of altering central serotonin (5-HT) by mimicking (tryptophan), blocking (cinanserin) and depletion (PCPA), on morphine induced changes on the sensory field potential were examined. Permanent semimicroelectrodes constructed of 60μ nichrome wire were implanted stereotaxically under pentobarbital anesthesia within the caudate nucleus and substantia nigra in 30 Sprague-Dawley male rats weighing 200-300g. Animals were assigned randomly to the three groups of this experiment. After control recording of the sensory (acoustic) evoked potentials, group I rats were treated with morphine (10 mg/kg i.p.) and recordings were resumed for 45 minutes; the animals then were injected with naloxone (1 mg/kg i.p.) and recordings were resumed for another 45 minutes. On day two, once again control recordings were obtained, then the animals were treated with tryptophan (25 mg/kg i.p.) and recordings were resumed for 45 minutes. At this time, the animals received morphine (10 mg/kg i.p.) and recordings were again resumed for another 45 minutes. At the end of the experiment, animals were sacrificed and the brains were removed for histological verification of electrode placement. The procedure of group II was similar, but instead of tryptophan, animals were treated with cinanserin (15 mg/kg i.p.). In group III on day one of the experiment, after control and morphine recordings, animals received PCPA (300 mg/kg i.p.), and on day two of the experiment, after control recording, animals were treated with morphine (10 mg/kg i.p.) and recordings were resumed for 45 minutes. In general, the components of the sensory evoked responses were altered significantly after morphine in 64% and 39% of the recordings obtained from caudate nucleus (CN) and substantia nigra (SN), respectively. Tryptophan treatment intensified the morphine effect in CN and tended to reverse the direction of the amplitude responses in SN. Cinanserin treatment had minimal effects on subsequent morphine actions in CN, while in SN morphine induced changes in the opposite direction, mainly decreases in the response amplitudes. PCPA (300 mg/kg i.p.) reversed the morphine effects in CN from mainly increased responses to almost uniform decreases. PCPA had little effect on morphine actions in SN. It seems likely that 5-HT participates in morphine effects in CN and possibly SN. (Supported by USPHS grant DA 00803.)

815 STUDIES ON POST-DEPRESSION EXCITATION AFTER ETHANOL IN RATS. Carlton K. Erickson and David W. Grauer*. Dept. Pharmacol. Toxicol., Sch. Pharm., Univ. Ks., Lawrence, KS. 66045.

Earlier work has shown that small doses of ethanol (ETOH) can produce post-depression excitation or only stimulation of spontaneous locomotor activity in mice and rats (Erickson et al., Finn. Found. Alc. Stud. 23:177, 1975; Carlsson et al., Psychopharm. 26:307, 1972). To study this phenomenon further, rats were trained to perform in a discriminated lever press avoidance situation. Various intraperitoneal (ip.) doses of ETOH were given to rats responding at different levels of avoidance efficiency, and behavior was measured during and immediately after intoxication. "Moderately intoxicating" doses (e.g., 2.0 g/kg ip.) produced a depression of learned behavior (% avoidance) for approximately 2 hrs., followed by a return to control levels. Lever-pressing rates, however, were not depressed as dramatically during the action of ETOH, and "rebounded" above pre-drug levels after the depression of avoidance produced by ETOH. This effect was more pronounced in animals which were less-efficient responders (i.e., had lower mean % avoidance behavior). It is suggested that lever pressing behavior during avoidance responding may be a sensitive index for measuring post-depression excitation produced by ETOH. (Supported by USPHS Research Grant No. AA-01417 from NIAAA.)

816 IN VITRO EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON GANGLIONIC TRANSMISSION IN RAT SUPERIOR CERVICAL GANGLIA. James E. Forbes*, Miguel Lujan* and William L. Dewey, Dept. of Pharmacology, Med. Col. of Va., Richmond, Va. 23298.

It has previously been reported that Δ^9-tetrahydrocannabinol (Δ^9-THC) blocks the release of acetylcholine in the stimulated guinea pig ileum. Therefore, we studied the effects of Δ^9-THC on electrically evoked postganglionic potentials in rat superior ganglia in vitro. Adult male albino rats weighing 525-550 gms were anesthetized with pentobarbital (30 mg/kg, i.p.), the ganglia were dissected from the surrounding tissue, care being taken to keep the blood supply to the ganglia intact until the ganglia were removed. The superior cervical ganglia together with the preganglionic sympathetic trunk and external and internal carotid artery nerves were placed in a chamber, bathed with Eccles-Krebs solution, oxygenated with 95% O_2 - 5% CO_2 at a pH of 7.2 and a temperature of 36-38°C. Postganglionic potentials were evoked by electrical stimulation of the preganglionic sympathetic trunk with a biphasic supramaximal stimulus of 0.5 msec duration at a frequency of 1/sec. The evoked postganglionic potentials were recorded from the internal or external carotid artery nerve with a suction electrode. The Δ^9-THC was prepared in bovine serum albumin which gave negative results in vehicle tests. The effects of Δ^9-THC were biphasic on a temporal basis. Fifteen min after the addition of Δ^9-THC (3.3×10^{-4}M - 1.3×10^{-3}M), there was a 15% increase in the height of the potentials. This was followed by a dose related inhibition of the potentials over a 45 to 70 min period. The inhibition was characterized by both a decrease in the height and a slowing of the potentials. The Δ^9-THC concentration for 50% inhibition of the potentials was 1.3×10^{-3}M. The potentials returned to control levels following wash-out of the Δ^9-THC. (Supported by USPHS Grants DA00326, DA00450 and PSH Grant DE00116.)

817 THALAMIC EVOKED POTENTIALS IN NAIVE, TOLERANT AND WITHDRAWN RATS. Philip L. Gildenberg, K.S.Krishna Murthy, Martin W. Adler[1], and Elizabeth A. Frost*[2]. Div. Neurosurgery, Univ. of Texas Med. Sch. at Houston, Houston, Texas 77030 U.S.A.

Evoked potentials were recorded in the parafascicular and centrum medianum nuclei in male Sprague-Dawley rats under chloralose and light sodium pentobarbital anesthesia. Contralateral sciatic nerves were stimulated at both non-noxious and noxious strengths. In addition to naive rats, recordings were made in rats which had been previously made tolerant by repeated intravenous infusions of morphine over a 4-day period using a schedule which increased the dose every day. Recordings were also made from a third group of rats which, in addition to being made tolerant to morphine by the forced infusion schedule, were also in a state of withdrawal for a minimum period of 24 hours. The evoked potentials could be divided into an initial short latency burst of multi-unit activity followed by slow diffuse waves representing multisynaptic pathways. In the naive animal, the initial burst of multi-unit activity is reduced markedly after the administration of a single dose of morphine. In the tolerant animal, however, this reduction is less. The late slow diffuse wave becomes more positive on morphine administration in the naive animal. The slow response changes in latency and character during the development of tolerance. However, in the animal undergoing withdrawal, the slow wave again becomes positive.

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818 HEXADECAPEPTIDE α-ENDORPHINE: CENTRAL EFFECTS ON MOTOR FUNCTION, EEG AND SLEEP-WAKING CYCLE. Viktor Havlicek, Milan Rezek* and Henry G. Friesen*. Dept. of Physiol.,Univ. of Manitoba, Winnipeg, Man., Canada.

The central effects of a newly identified and purified pituitary hexadecapeptide α-endorphin (Endo:H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Oh, synthesized in Dr. R. Guillemin's laboratory) were investigated following its intracerebroventricular administration via chronic cannulas (1.0 and 10.0 μg/10μl) in freely-moving rats. The results were compared with the effects of control saline (10μl) and morphine (10μg/10μl) administered via the same route. Overall behavioral activation characterized by sniffing, horizontal and vertical exploration and accompanied by manifestations of facial and general body tremor, shaking, jerking, Straube tail phenomenon and frequent motor coordination difficulties (evidenced by impaired digit grip on the bars of the grid floor affecting the balance) was observed with varying intensity and duration after the infusion of morphine and with both doses of endorphine. In addition, early after the administration of morphine a paroxismal EEG activity was observed in 40% of the animals; in one case full EEG and behavioral seizures (Jacksonian type) developed. The administration of all three experimental substances also altered the composition of the sleep-waking cycle (as compared to saline controls): the awake state was prolonged (Endo 1μg, 23%; Endo 10μg, 51%; morphine 10μg, 58%) while substantial reductions in the duration of both deep SWS (Endo 1μg, 8.8%; Endo 10μg, 24.6%; morphine 10μg, 49.0%) and especially of REM sleep (Endo 1μg, 31%; Endo 10μg, 63%; morphine 10μg, 45% were observed. The present results do not provide evidence for a sedating effect of endorphine whose potential analgesic effects are now being tested in our laboratory.

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819 ETHANOL DEPENDENCE IN THE RAT: EXTRAPYRAMIDAL MOTOR SYSTEM INVOLVEMENT DURING WITHDRAWAL. Bruce E. Hunter*, Joseph N. Riley*, and Don W. Walker. Dept. Neuroscience, Coll. Med., Univ. of Fla. and VA Hospital, Gainesville, Fla. 32610.

Rats chronically implanted with electrodes in the caudate nucleus, red nucleus, substantia nigra and pontine reticular formation were maintained for 20 days on an ethanol-containing liquid diet. The removal of ethanol resulted in the time-dependent appearance of a variety of behavioral withdrawal symptoms including tail stiffening and arching, ataxia, rigidity, tremor, and spontaneous convulsions. Auditory-induced convulsions were elicited 8-12 hours post-withdrawal. The behavioral symptoms were rated according to a scale of withdrawal intensity developed for the rat. Electrographic recordings in conjunction with behavioral observations indicated the presence of severe epileptiform abnormalities including isolated spiking, bursts of organized spike activity, and periods of sustained seizure discharges. The epileptiform activity included periods of prolonged localized seizures in the absence of concomitant behavioral symptoms. The results suggest a more important role than heretofore anticipated for extrapyramidal motor structures in the ethanol withdrawal reaction.

820 DIFFERENTIAL EFFECTS OF MORPHINE ON SELF-STIMULATION FROM CLOSELY ADJACENT BRAIN REGIONS. J.M. Liebman and D.S. Segal. Dept. Psychiat., Sch. Med., UCSD, La Jolla, CA. 92093.

A previous investigation (Liebman and Segal, 1976) revealed unexpected heterogeneity in the effects of morphine (M) on self-stimulation (SS). We therefore examined the effects of chronically administered M on SS from various electrode placements in the substantia nigra (SN) area and the mesencephalic central gray (CG) region. Before pharmacological testing, current intensity was reduced to yield stable, submaximal rates of SS (400-1000 lever-presses/30 min). Rats were injected daily, s.c., with M for 10 days (10 mg/kg on Day 1, 12.5 mg/kg on Day 2, and 15 mg/kg thereafter). Between Days 5 and 10 of treatment, many rats self-stimulated at 150-250% of baseline but others reduced SS to as little as 3% of baseline or failed to alter responding. Histological evaluation revealed that M facilitated SS when the electrode tip was located more than 0.3 mm from the SN or more than 0.2 mm from the midline of CG. In rats with electrode tips closer to the SN, M generally reduced or failed to alter SS. It seemed possible that the facilitation of SS by M might be related to its blockade of aversive qualities accompanying SS from some brain regions. Therefore, the effects of chronic M were investigated on shuttle-box escape from stimulation of dorsal lateral tegmentum. Escape was reduced by M but this effect appeared to be unrelated to facilitation of SS because the respective time courses were quite different. Possible substrates of these effects are discussed.

821 MORPHINE EFFECTS ON CENTRALLY AND PERIPHERALLY EVOKED MULTIPLE-UNIT ACTIVITY. M. R. Palmer* and W. R. Klemm. Dept. Biology, Texas A&M University, College Station, Texas 77843.

Opiates have major effects on analgesia and on stereotypical movements; these effects are commonly evaluated in various behavioral tests, which are sometimes confounded by nonspecific performance variables. As a relatively unambiguous alternative physiological approach, we have tested the effects of morphine on electrical stimulation thresholds for evoking an increase in multiple-unit activity (MUA). Bipolar recordings from the caudate and central grey were made from curarized, artificially respired rats in which the sciatic nerve and the substantia nigra were stimulated. Morphine increased stimulus thresholds for sciatic evoked responses in both areas, with especially marked effects in the central grey. Morphine did not elevate thresholds for substantia nigra evoked responses in either area. Tonic (sustained) responses were suppressed more effectively than phasic responses, and the effect of morphine was greater 30 to 45 minutes after morphine injection than it was 5 to 15 minutes after the injection. Naloxone reversed all morphine effects, but did not change thresholds when used alone. (Supported by NIDA grant number DA 00784).

822 ANODYNIN: A NOVEL ENDOGENOUS OPIATE ANALGESIC ISOLATED FROM HUMAN BLOOD. Candace B. Pert, Agu Pert and John F. Tallman. Section on Biochemistry, Adult Psychiatry Branch, NIMH, Bethesda, MD. 20014.

A low molecular weight substance has been purified from human plasma on the basis of its ability to inhibit specific opiate receptor binding in rat brain membranes. This substance, termed anodynin, causes a profound long-lasting analgesia after microinjection into rat periaqueductal gray matter. The analgetic response, which was assessed by the "tail-flick" test, was significantly prevented by prior injection of the opiate antagonist naloxone. Anodynin is resistant to enzymatic degradation for one hour while the enkephalins (Hughes et al. Nature, 258, 577, 1975) show complete loss of opiate receptor affinity after a 10-min incubation with washed brain membranes. This is consistent with the finding that enkephalin-elicited analgesia is demonstrable for only three minutes after injection while anodynin-elicited analgesia is present for over one hour. One week after hypophysectomy, the levels of opiate receptor inhibitory activity in rat serum were reduced to less than 4% of levels in sham-operated control rats. We suggest that anodynin is a morphine-like hormone released into blood from the pituitary gland or requiring a pituitary factor for its maintenance.

823 EFFECTS OF ENKEPHALIN ANALOGS ON THE GUINEA PIG ILEUM. Margarita M. Puig*, Pedro Gascon*, Gale L. Craviso*, Richard A. Bjur*, John M. Stewart* and José M. Musacchio. Dept. Pharmacol., NYU Med. Ctr., New York, N.Y. 10016, and Dept. Biochem., U. of Colorado Sch. Med., Denver, CO. 80220.

Two naturally occurring pentapeptides, leucine-enkephalin (Leu-E) and methionine-enkephalin (Met-E), have been described by Hughes et al. (Nature 258: 577, 1976) to have morphine-like activity in the guinea pig ileum and in the mouse vas deferens.

We have synthesized Leu-E and Met-E as well as several analogs in order to study their relative potency on the guinea pig ileum and their mechanism of inactivation by different tissues. The ID_{50} of the various analogs was determined in the guinea pig ileum and compared to that of morphine (M). The relative potency of these analogs compared to M (M=1) was found to be: Met-E = 1.8; Met-E amide = 2.8; Leu-E and the sulfonium analog of Met-E = 0.2-0.3; 3-benzyl-tyrosine analog of Met-E = 0.03. The action of the 3-benzyl-tyrosine analog of Leu-E was negligible.

The rate of inactivation of Met-E, Met-E amide and Leu-E was found to be similar in the guinea pig ileum and in the brain opiate receptor membrane preparation.

(Supported by PHS-NIMH grants DA00351 and MH-17785).

824 DIFFERENTIAL EFFECTS OF MORPHINE ON RATES OF TRANSLATION OF SECRETED AND RETAINED PROTEINS IN RAT BRAIN REGIONS. James C. Ramsey* and William J. Steele. Dept. of Pharmacology, University of Iowa, Iowa City, IA 52242.

In vivo rates of translation of secreted and retained proteins were measured in 5 brain regions, whole brain and liver during the development of physical dependence on morphine to assess the participation of translation independently of transcription in dependence development. Rats were implanted subcutaneously with morphine (75 mg) or placebo pellets. After 1, 2, and 3 days, they were divided into two groups and either pulse-labeled with ^{3}H-leucine or injected with naloxone (0.4 mg/kg). The degree of dependence was assessed by counting the number of wet dog shakes occurring within the first 15 min after naloxone injection. The rate of translation (transit time) was determined by measuring the incorporation of label into total protein (t) and nascent polypeptide chains on polysomes (n) in the two compartments and graphing ratios of t/n versus time. Morphine increased the rate of translation of secreted proteins in the diencephalon, mesencephalon and pons-medulla but not in the cortex and cerebellum, whereas it increased the rate of translation of retained proteins only in the diencephalon. In contrast, morphine had no effect on the rate in either compartment of whole brain or liver. The increases in the rate of translation of secreted proteins in the pons-medulla were directly correlated to the development of dependence on morphine, suggesting that the latter occurs by means of a compensatory increase in the rate of synthesis of secretory proteins and/or proteins destined for the external surface of the cell. (Supported by USPHS grant DA 00710.)

825 MORPHINE ACTION ON THE RABBIT PUPIL AS A FUNCTION OF LIGHT LEVEL. R.J. Tallarida*, R. Kester*, H. Klemfuss*, M.W. Adler. Temple Univ. Sch. of Med., Philadelphia, Pa. 19140.

In the unanesthetized rabbit, morphine (12-16 mg/kg i.v.) produces a miosis that is not sustained. The response is a pupillary constriction that becomes maximal within two minutes, after which the pupil size fluctuates appreciably. The magnitude of the fluctuation is typically as high as 50% of control diameter and its frequency range is 0.5-2/min. (A film will be shown of this effect.) The miosis and subsequent fluctuation resulting from 12-16 mg/kg of morphine is blocked by naloxone (0.5 mg/kg i.v.), but 128 mg/kg of morphine overcomes the block. In order to explore the possible relationships between the actions of morphine and light on the pupil, experiments were conducted on the effects of morphine as a function of light level, utilizing filtered (red) light to which the rabbit retina is insensitive. Under such conditions, the response to morphine in dark-adapted rabbits is a decrease in mean diameter from 6.69 mm to 5.69 mm and a subsequent fluctuation, a response which is qualitatively similar to that seen in normal laboratory illumination. In rabbits which are not dark-adapted at the time of morphine administration, the narcotic produces an increase in mean diameter from 7.59 to 8.11 mm. In another set of experiments, animals in normal laboratory illumination were exposed to a pulse of high intensity (white) light, and at a time (5 min later) when pupil size had returned to control, i.v. morphine (12 mg/kg) produced a diminished response; i.e., less constriction than normal. These experiments support the contention that the pupillary effects of morphine result from stimulation of the pupilloconstrictor neurons in the visceral nuclei of the light reflex pathway. (Supported in part by USPHS grant DA00376 from NIDA).

826 EFFECTS OF REPEATED ELECTROCONVULSIVE SHOCKS ON THE ALCOHOL WITHDRAWAL SYNDROME. P.H. Van Oot* and J.P.J. Pinel. Dept. Psychol., U.B.C., Vancouver, B.C., Canada, V6T 1W5

There was a progressive increase in the severity of the convulsive response produced in rats by a series of 10 electroconvulsive shocks (ECSs) administered at 3-day intervals. The 10 distributed ECSs also produced an increased susceptibility to the convulsive effects of alcohol withdrawal following 2 weeks of forced exposure via gavage. Thus, the increased seizure susceptibility produced by a series of ECSs is, at least to some extent, general. This effect illustrates a potential hazard of electroconvulsive therapy.

In a subsequent series of experiments the effects of various parametric manipulations on this phenomenon were investigated. The progressive increase in the severity of the ECS-produced seizures and the potentiation of the alcohol withdrawal syndrome were both observed at either of two ECS intensities (15 or 75 mA) but only when the inter-ECS intervals were greater than 1 day. The potentiation of the alcohol withdrawal syndrome by 10 ECSs administered at 3-day intervals lasted at least 3 weeks, but no potentiation of alcohol withdrawal was produced at the 3-week interval unless 6 or more distributed ECSs were administered. In a final experiment rats received each of the 10 ECSs after receiving the series of drugs typically administered prior to clinical ECS treatments (atropine, barbiturate, succinylcholine, and oxygen). In these animals, withdrawal intensification was still produced, but to a slightly lesser degree than in subjects receiving no drugs.

Until the appropriate tests have been conducted on human patients, administration of potentially convulsive drugs should be carefully monitored after ECS treatments.

827 REVERSAL OF MORPHINE CATATONIA BY CENTRAL NALOXONE: A NEUROANATOMICAL ANALYSIS. Richard E. Wilcox*, Robert A. Levitt, Michael McCoy*, and Michael Bozarth*. Dept. Psych., Southern Ill. Univ., Carbondale, IL, 62901.

Mapping the brain for each major response is preliminary to determining if the behavioral effects induced by morphine are mediated by the same brain substrates. Initial mappings for loci mediating analgesia and physical dependence to morphine have been made. However, the sites modulating morphine-induced changes in motor function remain to be established. Studies on the motor actions of morphine would appear of value for two reasons. (1) They may help establish whether the behavioral effects of morphine are due to distinct neural substrates. (2) Motor system "side" effects of morphine may sometimes blur the measurement of other morphine actions, such as analgesia or withdrawal. The present study was a dose-response analysis of the ability of central naloxone to reverse a morphine-mediated motor action, catatonia. Each rat received 1 systemic injection of 80mg/kg morphine followed by 1 brain injection. Either naloxone or saline was administered centrally in 1 microliter of solution. Six naloxone doses were used, ranging from .001-100 micrograms. A total of 187 stimulations were made in 11 brain areas. Stimulation sites were classed as having high or low opiate receptor binding according to data for rat (Pert et. al., Life Sci 16:1849,1975), monkey (Kuhar et. al., Sci 245:447,1973) and human (Hiller & Simon, J Neurochem 20:1789,1973). Probability of reversal of the catatonic response after naloxone was significantly higher for stimulations in high binding areas than for low binding areas. A dose effect was found for naloxone. Results confirm the importance of central gray core in mediating morphine effects and implicate additional areas in modulating the actions of morphine-catatonia.

Neurochemistry

828 ADENYLATE CYCLASE RESPONSIVENESS TO CATECHOLAMINES IN THE SUPERIOR CERVICAL GANGLION OF VARIOUS SPECIES. Asa C. Black, Jr., Tanemichi Chiba, James K. Wamsley*, and Terence H. Williams. Dept. Anatomy, University of Iowa College of Medicine, Iowa City, Iowa 52242.

Small, intensely fluorescent (SIF) cells have been found in the superior cervical ganglia (SCG) of all mammalian species studied to date. Libet[1] and Greengard[2] have advanced the concept that at least some of these cells act as inhibitory interneurons by hyperpolarizing the principal ganglionic neurons through the generation of a slow inhibitory postsynaptic potential (s-IPSP). The action of a dopamine receptor--adenylate cyclase complex in this mechanism is crucial[2].

We have determined the effect of in vitro stimulation of adrenergic receptor--adenylate cyclase complex(es) with 50 μM concentrations of dopamine, l-norepinephrine, and l-isoproterenol on cat, cow, and guinea pig SCG incubated in vitro at 37 C in Eagle's Medium containing 5 mM theophylline[3].

Species	Dopamine[a]	l-Norepinephrine	l-Isoproterenol
Cat	41.0 ± 4.5(4)	40.0 ± 4.5(8)	28.1 ± 2.0(4)
Cow	151. ± 21(6)	118. ± 12(5)	103. ± 9.7(6)
Guinea Pig	31.0 ± 1.9(4)	47.1 ± 5.6(7)	217. ± 18(5)

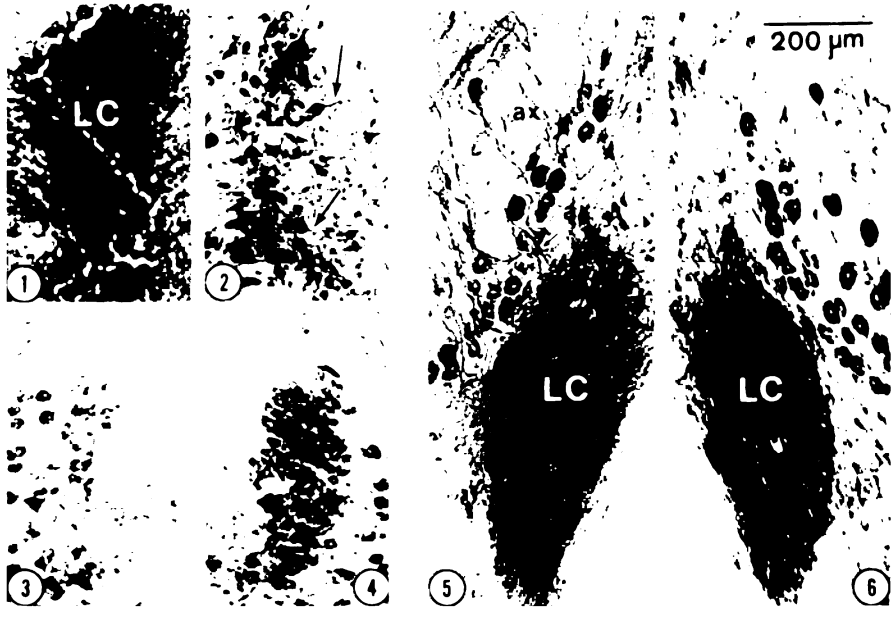
[a]All values in picomoles cyclic AMP per mg. protein ± Standard Error of the Mean; number of samples in parenthesis. Control values were 26.9 ± 4.9(4)(cat), 28.8 ± 2.2(6)(cow), and 30.0 ± 2.9(4)(guinea pig).

Interpretation: The bovine SCG contains both a dopamine receptor--adenylate cyclase complex (located on the postsynaptic membrane of the synapse of the SIF cell-interneuron with the principal ganglionic neuron[2]), and a β-adrenergic receptor--adenylate cyclase complex (unrelated to interneurons[4,5,6]). The guinea pig SCG contains only the β-adrenergic receptor--adenylate cyclase complex, while the cat SCG contains only a small amount of the dopamine receptor--adenylate cyclase complex.

We now have evidence that: 1. In the cat, Type I SIF cells (presumed interneurons) are scarce[7,8], while a response to dopamine stimulation is relatively weak, and the s-IPSP in the cat is weak or erratic[9]. 2. In the cow, Type I SIF cells are abundant[7,8], and there is a strong stimulation of adenylate cyclase by dopamine. 3. In the guinea pig, the picture is unique: (a) differentiation of SIF cells into Types I and II could not be made[7]; (b) the response to dopamine is absent; (c) the response to β-adrenergic stimulation is considerable; (d) the SIF cell catecholamine was shown by Elfvin, et al.[10] to be norepinephrine; and (e) there is no s-IPSP in this species[1]. Supported by General Research Support from the Iowa College of Medicine and the Iowa Academy of Science. References: 1. Fed. Proc., 29:1945 (1970). 2. Fed. Proc., 33:1059 (1974). 3. Black, et al., in press. 4. J. W. Kebabian, Ph.D. Thesis, Yale University, 1973. 5. J. Pharm. Exptl. Therap., 188:676 (1974). 6. Science, 190:157 (1975). 7. Nature, 256:315 (1975). 8. Non-Striatal Dopaminergic Neurons: Fourth International Sardinian Symposium, May 24-28, 1976, in press. 9. N.-S. Arch. Pharmacol., 281:119 (1974). 10. J. Ultrastr. Res., 51:377 (1975).

829 EVIDENCE THAT ACETYLCHOLINESTERASE (AChE, EC 3.1.1.7) IS LOCALIZED WITHIN THE SOMATA, DENDRITES, AND AXONS OF NOREPINEPHRINE(NE)-CONTAINING NEURONS IN THE LOCUS CERULEUS (LC). Larry L. Butcher[1], Konrad Talbot[2], and Jane D. Curtis*[3]. Department of Psychology (1,2,3) and Brain Research Institute (1), University of California, Los Angeles, CA 90024, U.S.A.

LC displays intense AChE activity (fig. 1, transverse section). Individual neuronal somata and their processes are difficult or impossible to discern (fig. 1), presumably because the cell bodies are densely packed, and the AChE-containing processes overlap extensively. Using a pharmacohistochemical regimen (Butcher and Bilezikjian, 1975, *Europ. J. Pharm.* 34, 115; Butcher and Hodge, 1976, *Brain Res.* 106, 223; Butcher *et al.*, 1975, *Proc. West. Pharm. Soc.* 18, 25; Butcher *et al.*, 1975, *J. Neural Trans.* 37, 127), however, we have demonstrated that individual neuronal somata and processes in LC possess AChE (fig. 2, transverse section). The plaques of russet-colored reaction material are confined to a perinuclear array. The cell bodies are oval or round and usually have a single prominent process, possibly a dendrite, that stains for AChE (arrows, fig. 2). The distribution and morphologies of AChE-containing LC neurons are similar to those of LC neurons possessing NE (cf. Jones and Moore, 1974, *J. Comp. Neurol.* 157, 43), and, on a statistical basis alone, we suggest that neurons containing NE also contain AChE. Furthermore, retrograde degeneration of AChE neuronal somata in LC (fig. 3, compare with fig. 4 from same brain section on side opposite lesion; transverse sections) appears to be produced in rats having unilateral radio-frequency ablations in both the cerebellum and dorsal tegmental bundle, two areas possessing NE-containing afferents from LC. Finally, injection of colchicine into the dorsal tegmental bundle and cerebellum produces build-up of AChE within processes, probably axons (ax), emanating from LC cell bodies (fig. 5, compare with fig. 6 from same brain section on the side opposite the injection; horizontal sections). These data suggest that AChE is localized within all components (i.e., somata, dendrites, axons) of NE neurons in LC. Why this may be the case is open to speculation, but perhaps the hypotheses of Burn and Rand and of Nachmansohn should be considered seriously in this regard. Furthermore, AChE may be contained within LC cell bodies and dendrites to inactivate acetylcholine released from cholinergic afferents to LC. (Support: USPHS NS-10928 and Scott Fund)



830 DO NEURONAL SOMATA IN THE B7 5-HYDROXYTRYPTAMINE (5-HT) CELL BODY GROUP ALSO CONTAIN ACETYLCHOLINESTERASE (AChE, EC 3.1.1.7)? Stuart M. Deikel[1] and Larry L. Butcher[2]. Department of Psychology (1,2) and Brain Research Institute (2), University of California, Los Angeles, CA 90024, U.S.A.

Using various histochemical procedures, several investigators have reported that certain raphe nuclei in the brainstem stain for AChE (e.g., Ramon-Molinar, 1972, *Erg. Anat. Entw.* 46, 1; Palkovits and Jacobowitz, 1974, *J. Comp. Neurol.* 157, 29). In these earlier investigations, however, it was not possible to localize the reaction product reliably to a particular subcellular component of the neurons in the raphe regions. For example, Palkovits and Jacobowitz (1974, *op. cit.*) report that the vast majority of AChE in the dorsal raphe nucleus is associated with "processes"; similarly, these same authors observe no AChE-containing neuronal somata in the central superior nucleus (i.e., median raphe nucleus). Using a pharmaco-histochemical regimen for the demonstration of AChE (Butcher and Bilezikjian, 1975, *Europ. J. Pharm.* 34, 115; Butcher and Hodge, 1976, *Brain Res.* 106, 223; Butcher *et. al.*, 1975, *Proc. West. Pharm. Soc.* 18, 256; Butcher *et. al.*, 1975, *J. Neural Trans.* 37, 127), however, we have been able to demonstrate that the AChE within the dorsal raphe nucleus (DR, fig. 1), median raphe nucleus (MR, fig. 2), and raphe magnus nucleus (RMa, fig. 3) is associated, at least in part, with discrete neuronal somata and their processes. Of particular interest is the localization of AChE within dorsal raphe cell bodies, since comparison of the distribution and morphologies of AChE-containing neurons in this region with those dorsal raphe neuronal somata containing 5-HT reveals considerable similarity (e.g., compare fig. 1 with fig. 36 in Dahlström and Fuxe, 1964, *Acta Physiol. Scand., Supplem. 232* 62, appendix). Although correlations between AChE- and 5-HT-containing neurons in the raphe magnus and median raphe areas is not clear at present, it is possible that AChE is contained within 5-HT neurons in the dorsal raphe nucleus, perhaps to inactivate acetylcholine released from cholinergic afferents. (Support: USPHS NS 10928 and Scott Fund)

831 A COMPARISON OF THE EFFECTS OF MORPHINE AND NALOXONE ON INCORPORATION OF ^{3}H-l-LYSINE INTO VARIOUS NEURONS IN WISTAR AND STRAGUE DAWLEY RATS.

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Male Wistar and Sprague Dawley rats were pretreated with 40 mg/kg of morphine sulphate 1 hour before receiving 2.92 to 3.50 ug/kg of ^{3}H-l-lysine via indwelling intravenous catheters while in an unanesthetized state. Rats were then killed at 15,30,45 and 60 intervals after the lysine injections. Some Sprague Dawley rats also received naloxone (10 mg/kg) 5 min. previous to morphine-treatment in an attempt to block morphine effects. Ventral horn neurons and Purkinje cells were separated from small blocks of formalin fixed gray matter by ultra sonification at low amplitudes in 10% sucrose at time intervals which varied with the nerve cell type. The sucrose suspension was then plated out and the neurons allowed to settle. The suspension was then washed with deionized water until most of the debree and glia were removed. Groups of 400 neurons were then collected and solubilized with NCS solubilizer (Amersham) after which a toluene-scintilator cocktail was added preparatory to liquid scintillation counting. Since prolonged formalin fixation removes free amino acids from tissue, the resulting counts represent mainly ^{3}H-lysine associated with neuronal protein and have been expressed as ng ^{3}H-lysine/gm dry weight of neuronal mass. With the Wistar rats, morphine produced a marked inhibition of incorporation of lysine into ventral horn and Purkinje cell neurons. In Sprague Dawley ventral horn neurons, there was no change in amino acid uptake caused by morphine and the time course of lysine accumulation was markedly different and much lower than in Wistar rats. In Sprague Dawley Purkinje neurons, morphine actually caused an increase in lysine accumulation 45 and 60 minutes after injection. Providing naloxone to the Sprague Dawley group elevated lysine accumulation in ventral horn neurons above control and morphine treated levels. Naloxone, has been previously reported to increase accumulation of lysine into brain protein (Ford, et al., in press). However, naloxone had no effect on lysine accumulation into Purkinje cells. The data demonstrated definite strain differences in the effect of morphine on accumulation of ^{3}H-lysine into neurons. It is also apparent that naloxone has different effects on lysine accumulation into different neurons, being stimulatory in ventral horn neurons and ineffective on Purkinje cells.

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832 GAMMA-HYDROXYBUTYRIC ACID (GHB): SELECTIVE ASSOCIATION WITH GABA-ERGIC NEURONAL SYSTEMS. Barry I. Gold*, John D. Doherty and Robert H. Roth. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

The in vivo formation of [^{3}H]GHB from [^{3}H]GABA has been reported (Roth & Giarman, Biochem. Pharmacol. 18:247, 1969), although it was concluded that this pathway was not normally very active. Recently, Tabakoff & von Wartburg (Biochem. Biophys. Res. Comm. 63:957, 1975) reported the existence of NADPH-coupled dehydrogenases, physically separable from lactate- or alcohol dehydrogenases, which catalyze the reduction of succinic semialdehyde (SSA), formed by the transamination of GABA with 2-oxoglutarate, to GHB.

Conscious male rats were given 15 μCi of [^{3}H]GABA via a cannula implanted in a lateral ventricle 24 hrs previously. Rats were sacrificed at various times after [^{3}H]GABA, and [^{3}H]GHB was extracted (as γ-butyrolactone) from trichloroacetic acid homogenates of whole brain from which [^{3}H]GABA had been removed by cation exchange chromatography. [^{3}H]GHB formation was found to be maximal 30 secs after [^{3}H]GABA. Amino-oxyacetic acid (AOAA, 50 mg/kg i.p., 60 min before [^{3}H]GABA) reduced [^{3}H]GHB formation, measured 4 min after [^{3}H]GABA, to 28% of that found in animals which had received [^{3}H]GABA and AOAA vehicle. This strongly suggests that SSA is indeed an intermediary metabolite in the GABA to GHB pathway. Significantly more [^{3}H]GHB could be recovered from rats which had received 50 mg/Kg i.p. of the convulsant 3-mercaptopropionic acid 20 min before [^{3}H]-GABA. This dose produced no significant change in whole brain GABA levels assayed by the method of Enna & Snyder (J. Neurochem. 26:221, 1976).

Samples of human caudate nucleus, globus pallidus, putamen, and frontal cortex obtained at autopsy were homogenized and aliquots of the homogenates were taken for assay of endogenous GABA, dopamine (DA) and GHB (method of Doherty et al., Anal. Biochem. 69:268, 1975). A significant positive correlation was found between GABA and GHB concentrations in these areas ($r = 0.95$, $p \leq 0.05$). No significant correlation was found between DA and GHB ($r = 0.76$), providing further evidence for an association of GHB with central GABA-ergic systems.

Further experiments are in progress to determine the regional distribution of the NADPH-coupled dehydrogenases which catalyze the reduction of SSA and whether the distribution correlates with GABA and/or GHB levels. (Supported in part by USPHS Grant #MH 14092 and Postdoctoral Training Grants #NS 05706 [B.I.G.] and #NS 05082 [J.D.D.].)

833 CATECHOLAMINERGIC EFFECTS ON ADENYLATE CYCLASE OF RAT STRIATAL AND LIMBIC FOREBRAIN SLICES. JoAnn Heltzel and Henry M. Sarau, Smith Kline & French Labs., Philadelphia, Pa. 19101

Various catecholaminergic agents were studied for their effect on cAMP production by rat striatal slices employing the method of Greengard (Science 186: 1118, 1974). Norepinephrine (NE), isoproterenol (ISO) and dopamine (DA) caused a dose related increase in cAMP produced. The EC_{50}'s (concentration causing 50% of maximum stimulation) were ISO, 0.5 μM; NE, 5 μM and DA, 500 μM. Maximum stimulation by NE and DA was greater than four fold above basal levels while the ISO maximum effect was only two fold. The DA effect was additive to the maximally effective dose of NE. Basal and NE (50 μM) stimulated activity was unaffected by 100 μM phenylephrine or phentolamine while 100 μM propranolol completely antagonized the NE increase suggesting β-adrenergic receptor properties. Antipsychotic drugs (chlorpromazine, thioridazine, fluphenazine and clozapine) did not significantly antagonize NE stimulation in striatal slices but partially blocked NE stimulation in limbic forebrain slices. DA (5 mM) stimulation was not affected by phenylephrine and only partially blocked by phentolamine and propranolol at equimolar concentrations and by chlorpromazine at 100 μM. Striata (innervated and denervated) from rats with unilateral nigro striatal 6-hydroxy-dopamine lesions that exhibited rotational behavior consistent with striatal denervation supersensitivity were tested for responsiveness to catecholaminergic agents. Slices of denervated striata were significantly more responsive to NE and ISO compared to innervated slices but no significant change to DA stimulation was obtained. Limbic forebrain slices ipsilateral to the lesion indicated no increased responsiveness to ISO, NE, or DA. Striatal slices from rats chronically treated with haloperidol had increased responsiveness to NE and ISO. Limbic forebrain slices from these animals also exhibited supersensitivity. The findings indicate the presence of β-adrenergic activity in the caudate slice; the preparation shows the induction of a supersensitive response to NE after denervation with 6-hydroxy-dopamine or with chronic haloperidol treatment. Another activity is observed in the preparation but it doesn't appear to be classically dopaminergic.

834 THE CHARACTERIZATION OF THE SODIUM-DEPENDENT HIGH-AFFINITY UPTAKE OF TAURINE INTO RAT BRAIN SYNAPTOSOMES. R.E. Hruska*, R. Bressler*, and H.I. Yamamura. Dept. Pharmacology, Col. of Med., Arizona Med. Ctr., Univ. of Arizona, Tucson, AZ 85724.

Recently, interest has been focused on the role of brain taurine in certain disease states as well as on its possible role as a neurotransmitter or neuromodulator. One of the mechanisms available for terminating the action of released taurine in the brain is its re-uptake into nerve endings. Several reports have appeared in the literature demonstrating such a transport system. Recently, we reported a unique sodium-dependent, high-affinity transport system into rat brain synaptosomes for taurine using tritium-labeled taurine of a high specific activity (Fed. Proc.35: 325). Using a microcentrifugation technique, we have characterized a sodium-dependent, high-affinity uptake for taurine. We removed whole rat brain, homogenized it in 0.32M sucrose, and used the crude synaptosomal fraction in subsequent studies. The tissue was added to microfuge tubes containing 1 ml of a buffered Krebs-Ringer medium. After preincubation at 37°C for 10 min, uptake was initiated by the addition of ^{3}H-taurine. The tissue was incubated for 4 min with varying concentrations of taurine. Then the uptake was terminated by centrifuging the tissue for 30 sec using a Beckman Microfuge B. The pellet was washed immediately, the microfuge tip was removed, and the tissue was extracted overnight in a Triton-toluene fluor at room temperature. Sodium-dependent uptake was determined by using a duplicate set of tubes containing a sodium-free medium. This medium was made by replacing the NaCl with LiCl and by replacing the sodium phosphate buffer with a Tris phosphate buffer at pH 7.4. Using this technique, we found that the initial rate of sodium-dependent, high-affinity uptake of taurine was linear for 4 min. The affinity constant (Km) for uptake was about 5-10 μM and the maximum velocity of uptake (Vmax) was about 30 nmole/g protein/min. The sodium-dependent, high affinity uptake of taurine is dependent on the existence of viable, intact synaptosomes. Uptake is reduced to 11% of control values in preparations lysed by freezing, thawing, and homogenizing on a Polytron. Optimal taurine uptake occurs in a range from pH 7.0 to pH 8.2, and is temperature dependent. At 3°C, there is no sodium-dependent, high-affinity uptake of taurine. At 25°C, uptake is reduced to 43% of that at 37°C. Taurine uptake is also reduced to 25% and 68% of control, respectively, in the absence of potassium or chloride ions. Altering the concentration of sodium, potassium, or chloride ions appears to affect primarily the velocity of transport without affecting the affinity of taurine for the transport carrier. The high-affinity, sodium-dependent uptake of taurine is markedly inhibited by the presence of 1 μM ouabain in the incubation medium. The amount of ouabain inhibition is dependent upon the amount of time that ouabain is preincubated with the tissue. Preincubating the tissue with ouabain for 10 min decreases taurine uptake to 41% of control, whereas a 15 min preincubation decreases transport to 25% of control. Therefore, taurine uptake has been characterized as a ouabain-sensitive, pH, temperature, and energy dependent system; and it is sensitive to ionic alterations under our in vitro assay conditions. Characterizing the sodium-dependent, high-affinity uptake of taurine may lead to an understanding of and a treatment for some of the neurological disorders in which taurine is purported to be involved. (Supported by USPHS Grants MH-26967 and MH-27257 and RSDA MH-00095.)

835 RAT BRAIN ARYL ACYLAMIDASE: FURTHER STUDIES ON MULTIPLE FORMS. Louise L. Hsu*, Angelos E. Halaris and Daniel X. Freedman. Dept. Psychiatry, Univ. of Chicago, Chicago, Il. 60637.

We have recently reported on the existence of multiple forms of rat brain Aryl Acylamidase (AAA) separated by Bio-Gel chromatography at pH 5.5 (Hsu et al., J. Neurochem., submitted for publication). We have also described the stereospecific inhibitory effects of LSD, serotonin and indolealkylamine-related compounds as well as the effects of pH on enzymatic activity of the two forms of AAA. Now, we present data on these different forms of AAA in brain by separating them from Bio-Gel column at pH 7.5. We also report on the effects of various drugs on at least two forms of AAA. In addition to LSD and serotonin, we studied psychotomimetic indolealkylamines (bufotenine and DMT) and phenylethylamines (d-amphetamine and mescaline), catecholamines (dopamine and norepinephrine), histamine and cAMP. Bio-Gel (A-5m) column chromatography was performed as previously described except that Na-phosphate buffer at pH 7.5 was used instead of pH 5.5. When the proteins were eluted from the column with the buffer at pH 5.5, a small sharp peak came off the column between fractions 20 and 25. A broader peak appeared between fraction 48 and 62. The column fractionation at pH 7.5 also resulted in two distinct protein peaks as it did at pH 5.5. However, at pH 7.5 peak 1 was broader and larger at the same position; peak 2 was slightly shifted to the left and was narrower and smaller. When assayed at pH 7.5, the eluted fractions showed an enzyme activity peak (AAA-1) between the two protein peaks. On the other hand, when assayed at pH 5.5 the eluted fractions showed an enzyme activity peak (AAA-2) corresponding to the second protein peak. In contrast to the results obtained from column elution at pH 5.5, only one enzyme activity peak predominated when the column was eluted at pH 7.5 and when the AAA activity was assayed at either pH 7.5 or 5.5. Apparently, manipulation of pH values could selectively differentiate and separate at least two forms of AAA activity by Bio-Gel column chromatography. Furthermore, at pH 7.5, AAA-1 was markedly inhibited by d-LSD (65% inhibition) and 2Br-LSD (61%); it was moderately inhibited by 5-HT (21%), bufotenine (17%), and l-LSD (16%); it was slightly inhibited by tryptamine (12%) and DMT (11%), but it was not affected by catecholamines, d-amphetamine, mescaline, histamine or cAMP. On the other hand, AAA-2 was markedly inhibited by d-LSD (54%) and 2Br-LSD (57%); it was moderately inhibited not only by the indolealkylamines bufotenine (17%), DMT (28%), and tryptamine (22%), but also by DA (19%), NE (20%), mescaline (17%), histamine (22%) and cAMP (15%); there was a slight inhibition by 5-HT, amphetamine and l-LSD. When AAA was assayed at pH 5.5, we observed a similar trend of drug-induced inhibition of either form of AAA activity. Thus, both forms of AAA are inhibited by d-LSD, 2Br-LSD, 5-HT and related compounds but only AAA-2 was inhibited by catecholamines, phenylethylamine-related drugs, histamine and cAMP. The physiological or pharmacological significance of these different forms of AAA in brain and their interrelations remain to be determined.

836 NEW EQUATIONS FOR DESCRIPTION AND ANALYSIS OF CARBOHYDRATE METABOLISM IN EXPERIMENTS WITH SPECIFICALLY LABELLED GLUCOSE. Martin G. Larrabee. Biophysics Dept., Johns Hopkins University, Baltimore, MD. 21218.

In order to gain information from the time course of isotopic equilbration in excised tissue, equations describing metabolism of specifically labelled glucose (G) through the pentose cycle (PC), the Embden-Meyerhof glycolytic path (EMP), and the citric acid cycle (CAC) were rigorously derived under the following assumptions: (1) G metabolism is in a steady state and follows standard textbook descriptions for nervous tissues, including infinitely repeated PC's and CAC's with the known reshuffling of C atoms within them. (2) Carbohydrate enters the PC only through the 6-phosphogluconate dehydrogenase (6PGDH) step. (3) Net syntheses of pentose and glycogen are negligible. (4) Delays of ^{14}C in intermediates are negligible, except in "pool a", which is common to EMP and PC before the 6PGDH step, and "pool p", which includes products of PC that receive no C-2 of G, such as dihydroxyacetone-P and fructose-diP. (Pool "p" explains why the output of labelled CO_2 rises more slowly with [^{14}C-6]- than with [^{14}C-1]- or [^{14}C-2]G in dorsal root ganglia from chicken embryos, even when the final steady state rates are about the same for all 3 (Wei-Kung Wang 1973).) (5) Traffic at entrances and exits of pools "a" and "p" is predominantly forward, letting the reactions be treated as irreversible. (6) The fraction of the products of the PC that are recycled into it is Rs for those that traverse pool "p" and Rf for those that do not. (Such recycling factors, suggested by Wei-Kung Wang (1973), avoid inapplicable assumptions about mixing of intermediates of PC and EMP that were made in the classic formulation of Wood et al. (1963).) (7) Carbohydrate that leaks from the PC is metabolized via the EMP.

The derivation included accounting for all routes to CO_2 and to leakage in the PC by 2 infinite sets of infinite series, leading to:

i $[1^*]CO_2 = Ka[1-\exp(-t/ta)]$ ii $Ka = A1+P1$

iii $[6^*]CO_2 = Kb[1-\exp(-t/ta)] + Kc[1-\exp(-t/te)]$

iv $Kb = A1 + P1[HRs+2F1(2-2Rf+H-HRs)]/[6-4Rf+HRs]$

v $Kc = 2P1[1-H][Rs(3-2Rf)+2F1(3-2Rf+RfRs-2Rs)]/[(6-4Rf-HRs)(6-4Rf-Rs)]$

vi $tp = te[6-4Rf-Rs]/[6-4Rf]$

vii $F1 = A1/[A1+L1+I(lip)1+I(aa)1]$

viia $A1/[A1+L1+I1] < F1 < A1/[A1+L1]$ viii $H = ta/(ta-tp)$

ix $A6 = A1 + 2F1P1[3-2Rf-Rs]/[6-4Rf-Rs]$ x $P6 = P1Rs/[6-4Rf-Rs]$

Where: t = time after adding [^{14}C]glucose. 1,6 designate C-1,C-6 of G. [*]CO_2 = rate of output of designated C of G to CO_2. A,P = steady state rates (s.s.r.) of output of designated C of G to CO_2 via CAC, PC respectively. ta,tp = time constants of pools "a","p". te = effective time constant of pool "p", increased above tp by recycling. Rs,Rf are defined above. H is arbitrary shorthand, defined by viii above. F1 = C-1 of G put out in CO_2 by CAC, as fraction of C-1 metabolized by EMP. L1 = s.s.r. of C-1 output to lactate. I1 = s.s.r. of C-1 incorporation into tissue. I(lip)1,I(aa)1 = s.s.r. of C-1 incorporation into lipids, amino acids, respectively.

In applications Ka, Kb, Kc, ta, and te are determined by curve fitting to data on [1*]- and [6*]CO_2. L1 and I1 are approximated by ave. rates for labelled C-1 in first few hours after adding labelled G. The equations can then be solved for all metabolic parameters, under the 2 extreme conditions of equation viia, for 2 special cases: (a) Rs = Rf, thus assuming equal leakage from both routes through PC, and (b) Rf = 1, thus assuming leakage only in the route through pool "p". By additional equations the outputs of C-6 to lactate and C-2 to CO_2 can be calculated: results agree reasonably well with observations. Derivations and applications will be shown. (Supported by NIH Grant NS09702.)

Refs.: Wang (1973) Dissertation, Johns Hopkins University, Baltimore MD.
Wood, Katz and Landau (1963) Biochem. Z. 338, 809-847.

837 BRAIN AND PERIPHERAL TRYPTOPHAN AVAILABILITY IN PROTEIN MALNOURISHED RATS. Maravene Miller*, Oscar Resnick*, J. Patrick Leahy* and Peter J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

The availability of tryptophan (Try) for serotonin (5-HT) synthesis from birth to 21 days in regional brain areas was examined in rats fed a normal (25% casein) or a low protein (8% casein) diet. The malnourished group, which received the low protein diet starting 5 weeks prior to conception, showed significantly elevated 5-HT and 5-Hydroxyindoleacetic acid (5-HIAA) levels at birth and at older ages as previously reported by Stern et al. (Exp. Neur. 49: 314, 1975). Also, these animals showed markedly elevated brain Try concentrations and plasma Try levels (total and free) at birth (table below). After day of birth, the total plasma Try levels of the malnourished rats were significantly lower than the control group. However, the malnourished animals showed consistantly greater percent free plasma Try concentrations and higher ratios of total brain Try to free plasma Try than the normal groups at all ages examined (day 5 to day 21).

Considerable evidence point to the theory that only the free plasma Try is available for 5-HT synthesis. The greater percent of free plasma Try, the higher ratios of brain to free plasma Try, and the elevated 5-HT and 5-HIAA brain levels in the malnourished rats is supportive of this theory. These results indicate that the malnourished rats may be shunting more Try into the brain probably as an adaptive response to protein malnutrition during gestation and lactation.

Day of Birth (Mean ± S.E.)

Diets:	8%	25%
(n)	8	8
	5-HT ng/gm	
Telencephalon	572 ± 32[a]	329 ± 32
Brainstem	783 ± 29[a]	462 ± 38
	5-HIAA ng/gm	
Telencephalon	789 ± 72[a]	524 ± 54
Brainstem	1,113 ± 41[a]	574 ± 46
	Try ng/gm	
Telencephalon	15,398 ± 1,213[a]	8,508 ± 598
Brainstem	16,190 ± 1,253[a]	9,163 ± 425
	Plasma Try ng/ml	
Total Try.	17,528 ± 825[a]	10,319 ± 319
Free Try.	16,967 ± 786[a]	9,626 ± 361
% Free	96.87 ± .62[a]	93.25 ± 1.08

[a] $p < 0.001$, 2-tailed t test

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838 SYNTHESIS AND SECRETION OF NERVE GROWTH FACTOR BY MUSCLE CELLS IN CULTURE. Richard A. Murphy, Robert H. Singer*, Judith D. Saide*, Muriel H. Blanchard*, Barry G. W. Arnason, and Michael Young*. Laboratory of Physical Biochemistry and Depts. of Biological Chemistry, Medicine, and Neurology, Harvard Medical School and Massachusetts General Hospital, Boston, Mass. 02114, and Dept. of Anatomy, Univ. of Massachusetts Medical Center, Worcester, Mass. 01605.

Recent studies from this laboratory have shown that nerve growth factor (NGF) is secreted by a variety of normal cells and transformed cell lines in culture. The physiological reason for NGF's secretion by these cells is not known; it could be that non-neuronal cells secrete the protein in order to communicate with elements within the nervous system. This line of reasoning led us to ask whether muscle cells secrete NGF. We find that primary rat skeletal muscle cells as well as the rat muscle-derived L_8 line synthesize and secrete a molecule immunochemically similar to mouse submaxillary gland NGF both before and after fusion.

Thigh muscles from newborn rats were trypsin dissociated. Unfused cells were prepared by incubating the tissue dissociates for 24 hours in serum-containing medium followed by a 48 hour period in serum-free medium. Myotubes were prepared by allowing the cells to fuse and then treating cultures with cytosine arabinoside for 48 hours. The myotubes were then incubated in fresh serum-free medium for an additional 48 hours. The L_8 line of rat thigh muscle cells were cultured as described above except that cytosine arabinoside was not used in fused preparations.

After the serum-free incubation period, conditioned medium was concentrated by lyophilization and its NGF content determined by radioimmunoassay employing mouse submaxillary gland NGF as reference standard. Assayed also were soluble fractions from cell homogenates. Immunoreactive material was found both intra- and extracellularly and increased in amount over time in both fused and unfused preparations. Concentrated culture solutions also stimulated a biological response indistinguishable from that seen with mouse NGF in the sensory ganglion assay system.

While the physiological significance of these findings is not clear, the results may be relevant to the nerve-muscle relationship and to nerve regeneration.

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839 HVA, ISOHVA, DOPAC AND p-HYDROXYPHENYLACETIC ACID (pHPAA) LEVELS IN THE URINE, PLASMA, WHOLE BRAIN AND CAUDATE OF THE RAT AFTER CHRONIC L-DOPA ADMINISTRATION. N. Narasimhachari, Robert C. Smith and John M. Davis, Illinois State Psychiatric Institute, Chicago.

It has recently been reported (Biochem. Pharmacol. 24, 43, 1975) that increased levels of isoHVA (up to 50% of HVA) were found in rats receiving L-DOPA and this was considered as the specific property of this species for increased paramethylation of dopamine. However the authors used a non-specific paper chromatographic method for the quantitation of HVA and iso-HVA. We have therefore repeated the experiments on L-DOPA loading with the rats and quantitatively determined the levels of HVA, isoHVA, DOPAC and pHPAA in the urine, plasma whole brain and caudate using the specific gas chromatographic mass spectrometric method described previously (Clin. Chim. Acta. 50, 337, 1974; 62, 245, 1975).

Male Sprague Dawley rats were injected I.P. with L-DOPA beginning at a dose of 10 mg/kg and increasing to a dose of 100 mg/kg over a six weeks period. DOPA rats showed no increase in stereotyped behavior or locomotor activity (recorded on aminex activity counters) at any dose of DOPA. Three rats were kept in a metabolic cage and 24 hour urines collected in each dose interval. Rats were killed by decapitation 2 hours after the final dose of 100 mg/kg and the caudate of each rat was dissected out. Whole brains from three rats were also used for the determinations. Saline rats were used as the controls. Blood was collected after decapitation into heparinized tubes which was then centrifuged to obtain plasma. The methyl or ethyl esters of the acids and their trimethylsilyl (TMS) ethers were used for quantitation by GCMS selected ion monitoring technique. The compounds were also identified by recording the complete mass spectrum during GCMS.

There were significant increases in the levels of HVA, isoHVA and DOPAC in urine, whole brain, caudate and plasma after L-DOPA but no dose dependent increased ratio of isoHVA/HVA was found in any of the tissues or body fluids, this ratio being 0.0-0.045 in urine and brain and 0.10 in plasma. The ratio of DOPAC/HVA was in urine 1.5, in plasma 2.1, in whole brain .07 and caudate .09. Levels of pHPAA increased in the urine and plasma after L-DOPA compared to saline controls (urine - SAL = .61 µg/24 hrs.; DOPA 100 mg/kg = 1883 µg/24 hrs.). However, there was significant decrease in pHPAA in the whole brain and caudate (SAL = 1.12 µg/gm; DOPA = .10 µg/gm). Thus while there was evidence for the dehydroxylation of dopamine in the periphery no such dehydroxylation is indicated in the central nervous system. Thus our results differ from previous reports. (Can. J. Biochem. 48, 1287, 1970). Higher levels of L-DOPA in the brain may inhibit the pathways of tyrosine metabolism which may account for the lower levels of pHPAA. In preliminary experiments with two monkeys given a single dose of L-DOPA we have obtained similar results. Thus we have not so far encountered any abnormal p-methylation after L-DOPA administration in cat, rat, dog, monkey or human.

840 MONOAMINE OXIDASE ACTIVITY IN NERVOUS AND PERIPHERAL TISSUES OF APLYSIA.
Jorge L. Ribas, George N. Catravas, June K. Takenaga* and David O. Carpenter. Dept. Neurobiology, Armed Forces Radiobiology Research Institute, Bethesda, MD. 20014.

Termination of action of biogenic amines in biological systems is mediated either by re-uptake mechanisms or by metabolic degradation. In the nervous system of Aplysia several laboratories have failed to detect monoamine oxidase (MAO), in spite of evidence that several biogenic amines function as neurotransmitters in this preparation. We have re-investigated this problem using a variety of substrates and report the presence of MAO in Aplysia. Aplysia californica, weighing approximately 300 g., were dissected and the kidney, liver, salivary gland, gill, heart, intestine and ganglia were homogenized in cold isotonic KCl or 0.25 M sucrose. MAO activity was measured according to the method of Wurtman and Axelrod (Biochem. Pharmacol. 12, 1439, 1964) using [^{14}C]-tryptamine as substrate. Highest MAO activity was found in the liver; appreciable amounts were also found in pooled ganglia, intestine, gill and kidney but salivary gland and heart had none. When [^{14}C]-tryptamine was used as substrate according to the method of Robinson et al. (Biochem. Pharmacol. 17, 109, 1968), the amount of MAO activity detected in the non-nervous tissues was at least as great as when tryptamine was used as a substrate. In the pooled ganglia, however, the MAO activity measured with tyramine was only 25% that determined using tryptamine. These results indicate that MAO is present in the nervous tissue of Aplysia and thus suggest that deamination may be an important pathway for the termination of action of at least some of the biogenic amines. There may, however, be differences in the MAO substrate specificity in different organs.

TISSUE	SUBSTRATE* TRYPTAMINE	TYRAMINE
Ganglia	1.453 ± 0.186 † (3)	0.365 ± 0.08 (2)
Liver	2.474 ± 0.023 (5)	3.022 ± 0.269(4)
Kidney	0.767 ± 0.027 (4)	1.986 (1)
Gill	0.214 ± 0.214 (2)	1.663 (1)
Intestine	0.382 (1)	
Salivary Gland	N.D. (2)	N.D. (2)
Heart	N.D. (1)	N.D. (1)

* Results are expressed as nmoles of substrate converted per mg. of protein per unit of time.

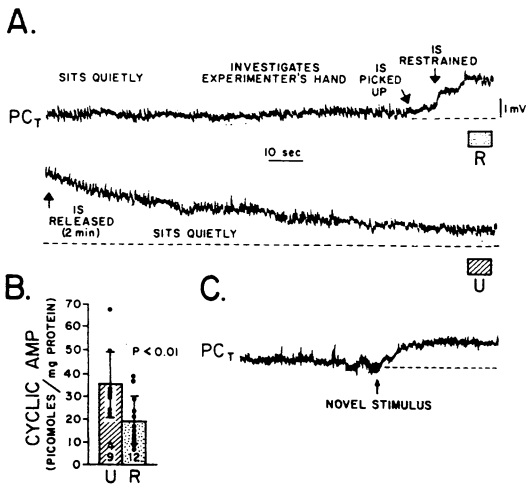
† Values are mean ± S.E.

N.D. None detected

() Number of experiments in duplicate

841 CORRELATED SHIFT IN A SURFACE-NEGATIVE SLOW POTENTIAL AND A REDUCTION IN 3', 5'-ADENOSINE MONOPHOSPHATE IN THE CORTEX OF CONSCIOUS RATS. J. E. Skinner, J. C. Reed*, K. M. A. Welch, and J. Nell*, Physiology and Neurology Depts., Baylor Coll. of Med. and Neurophysiology Dept., Methodist Hospital, Houston, TX. 77030.

Cyclic nucleotides have been implicated in the mediation of slow post-synaptic potentials in the sympathetic ganglia. The pharmacology of the specific neurotransmitters involved, the intracellular dynamics of the cyclic nucleotides activated and the resultant ionic effects that produce the membrane potentials have all been examined in the sympathetic ganglion model. That similar slow excitatory and inhibitory mechanisms are operational in the brain has been suggested by the demonstration that the local iontophoretic application of the transmitters and cyclic nucleotides results in slow increases and decreases in the firing rates of the nearby neurons. Extracellular cortical slow potentials of approximately the same durations as the effects on unit firing rate produced by the brief iontophoretic chemical stimulations can be recorded from the cortex of conscious animals during certain psychological conditions. Our hypothesis is that these slow potentials, like those in the sympathetic ganglia, are mediated by the cyclic nucleotides. In order to inactivate the cerebral tissue rapidly without evoking a cerebral slow potential we developed a method of rapid-freezing of cortical tissue (600° C/sec) by a chronically-implanted cryoplate. Slow potentials were recorded with platinum-black electrodes implanted next to the cryoplate. Surface-negative, depth-positive slow potentials were evoked in the parietal cortex of each rat by wrapping it in a towel. Unrestrained animals showed no slow potentials and a control level of 3', 5'-adenosine monophosphate of 35 picomoles/mg-protein, whereas restrained animals showed a slow potential associated with a reduction of 3', 5'-adenosine monophosphate to 20 picomoles/mg-protein ($P<0.01$). Since 3', 5'-adenosine monophosphate applications tend to cause cortical cells to stop firing, we conclude that our observed slow potentials and reduced levels of this cyclic nucleotide represent disinhibition of the underlying neurons.



A. Slow potentials recorded transcortically from parietal cortex (PC_T) before, during, and after forced restraint.

B. 3', 5'-adenosine monophosphate (cyclic AMP) levels in unrestrained (U) and restrained (R) rats.

C. Slow potential evoked by an unfamiliar sound.

842 THE CHARACTERIZATION OF MUSCARINIC CHOLINERGIC RECEPTOR BINDING IN THE HUMAN BRAIN. Gregory J. Wastek*, Lawrence Z. Stern , Peter C. Johnson*, Kevin Beaumont* and Henry I. Yamamura. Departments of Pharmacology, Neurology and Pathology, College of Medicine, University of Arizona Medical Center, Tucson, Arizona 85724.

The muscarinic cholinergic antagonist ^{3}H-quinuclidinylbenzilate (QNB) was used to characterize the kinetics of muscarinic receptor binding in autopsied human brain. Three brain regions (the putamen, the cerebral cortex and hippocampus) were used to examine QNB binding. Specific ligand binding to homogenates containing membrane fractions from the three regions was found to be linear up to 1 mg protein. As a consequence, tissue concentrations of about 0.2 mg protein per assay were routinely used in our studies. At 37°, specific QNB binding is half-maximal at about 5 min and plateaus by 40 min. However, all tissue samples were incubated for 60 min to insure equilibrium had been attained. The rate of dissociation of the QNB-receptor complex at 37°was extremely slow, $t_{\frac{1}{2}} \simeq$ 1-2 hrs. A large number of muscarinic antagonists and agonists effectively inhibited QNB binding. The antagonists were about 1000-fold as potent as the agonists in inhibiting QNB binding. Curves of QNB binding displacement by antagonists show Hill coefficients of 1.0, whereas curves showing displacement by agonists have Hill coefficients of about 0.5 suggesting negative cooperativity. Noncholinergic drugs were ineffective in displacing QNB binding.

Saturation kinetics for the three brain regions were measured at ligand concentrations between 0.02 to 1.8 nM. Specific QNB binding is saturable in the three regions whereas nonspecific QNB binding appears to be nonsaturable at these concentrations. Scatchard plot analysis yields dissociation constants (K_D) of 0.14, 0.09 and 0.07 nM with binding capacities of 774, 299 and 201 femtomoles/mg protein for the putamen, cerebral cortex and hippocampus, respectively.

Because the dissociation constants did not differ significantly among the three brain regions only one low concentration of QNB was used in subsequent examination of the regional distribution of QNB binding. Of the more than 30 brain regions taken from seven control human brains examined after autopsy, we found that ligand binding decreased when moving from the telencephalic regions (where we found the highest QNB binding) toward the brainstem and spinal cord regions (where QNB binding was barely detectable).

We also examined the regional distribution of QNB binding in the brains of three patients who died of Huntington's disease. We found significant reductions in ligand binding only in the caudate nucleus and putamen. Ligand binding in the putamen decreased from 471 $\pm$ 49 fm/mg protein in control brains to 124 $\pm$ 18 fm/mg protein in the choreic brains. In the caudate nucleus, QNB binding was 480 $\pm$ 16 fm/mg protein in controls and 172 $\pm$ 40 fm/mg protein in the choreic brains. Choline acetyltransferase activity in the two regions, as well as that in the globus pallidus, was also significantly reduced in the choreic brains whereas the protein values were unaltered. (Supported by U.S. Public Health Grants MH-26967, MH-27257 and RSDA, MH-00095 and a grant from the National Committee to Combat Huntington's Disease).

843 A RAPID METHOD FOR ASSAYING GABA-α-KETOGLUTARATE TRANSAMINASE-APPLICATION TO STUDIES OF DISTRIBUTION AND TISSUE-SPECIFICITY OF GABA-α-KETOGLUTARATE TRANSAMINASE. Jang-Yen Wu,* Larry G. Moss* and Obi Chude* (Spon: D. Louie). Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Previously, we had developed a rapid and specific method for assaying the GABA-synthetic enzyme, L-glutamate decarboxylase (GAD) (EC 4.1.1.15) (J. Neurochem. In Press). The method was based on the selectivity of ion exchange resin and the speed of vacuum filtration.

Since the substrates and the products of the GABA-degradative enzyme, GABA-α-ketoglutarate trasaminase (GABA-T) (EC 2.6.1.19), differ in charge, it should be possible to apply the rapid filtration technique with ion exchange resin for assaying GABA-T. The procedure is the same as those employed in GAD assay with the following modifications: BioRad AG1X8 was suspended in glass distilled water first, followed by 50mM glutamate, pH 2.8. The treatment of resin with glutamate is to saturate all the non-specific binding sites on resin which might trap labeled glutamate. The pH was chosen as to provide optimum retention of α-ketoglutarate and exclusion of glutamate. The retension of α-ketoglutarate was found to be virtually 100% when 0.7 to 5.1 u moles (1.5 X 10^5 to 1.1 X 10^6 CPM) of α-ketoglutarate were applied. The recovery of glutamate in the filtrate was found to be about 95% or better and was linear with the amount of glutamate applied in the range of 0.12 to 0.96 u moles (6.7 X 10^4 to 5.4 X 10^5 CPM) (Table I). In the actual enzyme experiment, GABA-T activity in mouse brain preparation was found to be linear with time up to 60 min. and with the amount of protein up to 1 mg. There are several advantages of this new assay method for GABA-T. First, this is a direct method, measuring the formation of product, glutamate, directly and hence, is most specific. Secondly, this is a rapid and easy method which makes it practical for assaying large number samples such as in the course of purification. Thirdly, it is also economical because the substrate, labeled α-ketoglutarate and the resin could be easily regenerated and reused. With this new method, GABA-T activity in various tissues has been determined as follows: brain, 8.14; spinal cord, 9.32; liver, 5.33; kidney, 4.48; lung, 0.16; heart, 0.13 u moles glutamate formed/min/mg. No GABA-T activity could be detected with muscle preparation. Since GABA-T has been purified to homogeneity from mouse brain in our laboratory and antibody against the purified GABA-T preparation also has been obtained [(Biochemistry 12, 2868 (1973); Brain Res. 65, 287 (1974)], it is feasible to compare GABA-T from variour tissues with immunochemical methods. GABA-T from brain, spinal cord and kidney are indistinguishable from the results of immunodiffusion, inhibition of enzyme activity by antibody and microcomplement fixation. It is, therefore, concluded that GABA-T from various tissues of mouse is probably identical or closely related to one another.

TABLE 1

α-Ketoglutarate Added (u moles)	Glutamate Added (u moles)	α-Ketoglutarate in Filtrate (u moles)	Glutamate in Filtrate	Recovery of Glutamate %
$\frac{0.7}{(1.5 \times 10^5 \text{ cpm})}$	--	0 (10 cpm)	--	--
$\frac{5.1}{(1.1 \times 10^6 \text{ cpm})}$	--	0 (48 cpm)	--	--
--	$\frac{0.12}{(6.7 \times 10^4 \text{ cpm})}$	--	$\frac{0.11}{(6.3 \times 10^4 \text{ cpm})}$	94
--	$\frac{0.24}{(1.3 \times 10^5 \text{ cpm})}$	--	$\frac{0.23}{(1.2 \times 10^5 \text{ cpm})}$	94
--	$\frac{0.48}{(2.7 \times 10^5 \text{ cpm})}$	--	$\frac{0.47}{(2.6 \times 10^5 \text{ cpm})}$	97
--	$\frac{0.96}{(5.4 \times 10^5 \text{ cpm})}$	--	$\frac{0.91}{(5.1 \times 10^5 \text{ cpm})}$	95

844 CYCLIC AMP-STIMULATED PHOSPHORYLATION OF A HIGH MOLECULAR WEIGHT ENDOGENOUS PROTEIN SUBSTRATE IN MOLLUSCAN NERVOUS SYSTEM. Eric Bandle* and Irwin Levitan (SPON: B. Gähwiler). Friedrich Miescher-Inst., P.O. Box 273, Basel, Switzerland.

Cyclic AMP stimulates incorporation of ^{32}P from ATP into endogenous phosphoproteins in a 20,000g supernatant prepared from Helix pomatia nervous system homogenates. There is no effect of cyclic AMP in the 20,000g pellet. Similarly cyclic AMP stimulates protein kinase activity in a 150,000g supernatant but not a 150,000g pellet. A part (but not all) of the cyclic AMP-stimulated activity in both supernatants is due to phosphorylation of an endogenous protein substrate, of apparent molecular weight 120,000 daltons as determined from its mobility in polyacrylamide gels. This protein incorporates little or no phosphate in the absence of cyclic AMP. Little phosphorylation of 120,000 dalton proteins is observed in particulate fractions, either in the presence or absence of cyclic AMP. When the 20,000g pellet is incubated with radioactive ATP and a partially-purified calf ovary protein kinase, cyclic AMP stimulates the phosphorylation of 120,000 dalton material. The data suggest that both membranes and cytosol from Helix nervous system contain 120,000 dalton substrates for cyclic AMP-dependent protein kinase. However no kinase catalyzing phosphorylation of these substrates is recovered in membrane fractions following homogenization of the tissue.

845 **MYO-INOSITOL AND OTHER SUGARS IN CEREBROSPINAL FLUID FROM PATIENTS WITH AFFECTIVE DISORDER.** **A. Barkai, H.A. Gross*, D.L. Dunner*, and R.R. Fieve*. New York State Psychiatric Institute and Department of Psychiatry, Columbia University, New York, New York 10032.**

The relationship between concentrations of free myo-inositol and its apparent precursor, D - glucose, in cerebrospinal fluid (CSF), was studied in hospitalized patients with primary affective disorder before and after administration of probenecid. Patients gave voluntary informed consent for all procedures. Myo - inositol, glucose and fructose were assayed simultaneously as trimethylsilylated derivatives by a gas chromatographic method which required minute aliquotes (20 µl) of lumbar CSF. The following mean concentrations of the sugars were obtained: Fructose - 22µg/ml, glucose - 600 µg/ml, and myo - inositol - 18 µg/ml. Probenecid administration (100 mg/kg orally in divided doses over a period of 18 hours) did not affect the concentrations of these sugars thus suggesting both that the drug does not interfere with cerebral synthesis of myo - inositol and that the transport of the sugars between CSF and blood is not sensitive to probenecid. Compared to values from two normal controls, the mean CSF level of myo - inositol but not of glucose or fructose appears to be lower in the depressed patients (mean level of myo - inositol in control CSF , 32 µg/ml). A linear relationship was established between the concentrations of myo - inositol and glucose in the CSF samples, indicating that the level of free myo - inositol in the CSF is dependent in part, on the level of glucose available as a precursor for its synthesis by the central nervous system. **(Supported by NIMH grants MH 21586 and NS - 11048.)**

846 COMPARTMENTATION AND RELEASE OF GLYCINE IN VITRO. P.M.Beart* and Khalidah B.Bilal* (SPON: E.T.Hedley-Whyte). Dept. of Neuroscience, Children's Hospital and Harvard Medical School, Boston, MA 02115.

Although glycine is considered an inhibitory transmitter in infraspinal regions of mammalian CNS, little is known of its metabolic regulation. The aim of the present study was to investigate the compartmentation and release of glycine by K^+ depolarization.

Slices of adult rat spinal cord (.5 x .5mm) were incubated in Krebs bicarbonate at 37° with either ^{14}C-glucose, pyruvate, serine or glyoxylate for 45 min. A large excess of 44mM K^+ or normal medium was added, the incubation continued for 5 min and slices and media separated by centrifugation. Both were analysed by the dansylation technique (Minchin & Beart, Br.Res., 83, 437, 1975). In further studies endogenous amino acid release was estimated by quantitative microdansylation (Beart & Snodgrass, J.Neurochem., 24, 821, 1975).

Endogenous glycine, glutamate and GABA were released by 44mM K^+, while serine was not significantly changed. ^{14}C-Glycine was present in all media, with the specific activity of K^+ released glycine being $>$ tissue glycine specific activity for glucose and serine, $\leq$ for glyoxylate and $<$ for pyruvate. GLN/GLU specific activity ratio was >1 with serine, glyoxylate and pyruvate, and <1 for glucose, suggesting non-nerve terminal and neuronal tissue metabolism respectively.

Both newly synthesized and endogenous glycine appear to be released by high K^+, just as for glutamate and GABA. Pyruvate appears to be a precursor for central glycine synthesis. Exogenous serine, glyoxylate and pyruvate may not be metabolized by neuronal mechanisms and may not mix with endogenous glycine stores, but may be important in vivo.

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847 THE EFFECTS OF L-METHIONINE AND/OR SHOCK STRESS ON THE BRAIN LEVELS OF INDOLEALKYLAMINES IN LONG-EVANS RAT BRAIN. John M. Beaton, Samuel T.Christian and Robert Harrison*. Neurosciences Program and Dept. Psychiatry, Univ. of Ala. in Birmingham, Birmingham, AL 35294.

It has been widely documented that L-methionine administration to schizophrenics induces an acute, florid psychotic reaction in some 40% of those tested. We have also shown that L-methionine disrupts the conditioned avoidance behavior of the rat. Stress is another factor thought to be important in the precipitation of schizophrenia. It has previously been shown that N,N-dimethyltryptamine (DMT) occurs in the synaptosomes of Sprague Dawley rat brain (Christian et al., Ala. J. Med. Sci., 13: 162, 1976). This present study was carried out to study the effects of methionine and/or shock stress, on the levels of DMT and other compounds in Long-Evans rat brain. Groups of rats were injected daily with saline or methionine (250 mg/kg) for 22 days. Half of these injected rats were placed, individually, one hour after injection, in a small restraining cage for 5 min per day on days 13-22. These rats were shocked on a fixed interval of 20 sec. On day 22 the rats receiving shock were killed immediately after the shock session. All other rats were killed 65 min after their last injection. Purified synaptic vesicles were prepared from groups of six rat brains. The vesicles were then extracted with methylene chloride and the extract derivitized with heptafluorobutyryl imidazole and then analyzed on a gas liquid chromatograph equipped with a ^{63}Ni electron capture detector. The preliminary results of this study indicate that O-methyl-bufotenin (OMB) is normally present in the rat brain and that the administration of L-methinine increases these levels. Shock stress on the other hand led to a marked increase in the levels of DMT.

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848 INACTIVATION OF LULIBERIN (LH-RF) BY HYPOPHYSEAL-PORTAL BLOOD OF PRIMATES. M.Benuck,* M.Ferin* and N.Marks. Res.Inst.Neurochem.,Rockland Res.Inst.,Ward's Island,N.Y.10035 and (M.F.) Internat.Inst.for Study of Human Reproduction, Columbia University, New York,N.Y.10032.

The stability of LH-RF in hypophyseal-portal blood is an important factor in its transport and biological half-life. Serum was prepared from blood obtained from the portal circulation of heparinized, ovariectomized, rhesus monkeys using the micro-surgical procedure of Carmen et al.(Endocrinol., 1976, in press). Blood was collected also from the femoral vein, and all samples were stored frozen. Separate studies with human sera showed no enzymatic changes when stored for several months.Breakdown was based on a biochemical procedure utilizing an autoanalyzer (M.Benuck and N. Marks, B.B.R.C. 65: 153, 1975) and results compared with those obtained by the radioimmunoassay procedure of Araki et al.(Endocrinol. 96:644, 1975). Analysis of breakdown products following 4-24 hr incubations at 37 C with the decapeptide showed a similar pattern of release for internal residues (Tyr^5, Leu^7) but a significantly lower rate for the C-terminal glycinamide by portal serum. Differences between portal and systemic serum were noted in amino acid composition, with portal serum containing a smaller pool of the major amino acids.

The results point to an internal cleavage by a neutral endopeptidase followed by secondary action of peptide hydrolases as previously proposed for tissue extracts.The rate of inactivation for monkey sera based on Tyr release was approximately six-fold lower than the rat, but similar to that previously obtained for human serum. The lower rate of LH-RF inactivation in primates, together with its rapid passage through the portal circulation, may be a significant factor in evaluating the endocrinological actions of LH-RF in man.

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849 ENDORPHIN OR MORHPINE ALTER BRAIN ADENYLATE CYCLASE ACTIVITY DEPENDING ON CALCIUM ION LEVELS. Kenneth A. Bonnet and Sara Gusik* Dept. Psychiat., New York University School of Medicine, New York 10016.

Narcotic agonists stereospecifically inhibit adenylate cyclase activity in neural tissues containing "opiate" receptors in some preparations when stimulated with prostaglandin E_1. The endogenous, narcotic agonist-like pentapeptide tyr-gly-gly-phe-met has been isolated from brain and shown to have analgesic-like effects in some preparations, as well. Like morphine, the endogenous or the synthetic form of the peptide has little effect on basal adenylate cyclase activity in vitro, but does block the PGE_1 stimulation of adenylate cyclase at 4 X 10^{-5}M. This block of the PGE_1 stimulation occurs in the presence or absence of2.5mM Ca^{2+} in the Krebs-bicarbonate medium. However, morphine in the presence of Ca^{2+} affected basal adenylate cyclase only at concentrations higher than 10^{-5} by stimulating activity; this concentration is much higher than that necessary in vivo to produce analgesia. Endorphin pentapeptide, in the presence of Ca^{2+} also stimulates brain adenylate cyclase in the range of 10^{-7} to at least 10^{-4} in a dose dependent manner.

Since one of the primary actions of narcotic analgesics is currently thought to be exerted through the regulation of adenylate cyclase activity in central nervous system structures, it is important that calcium injections have recently been reported to attenuate narcotic analgesia. We, and others have shown adenylate cyclase activity to increase with developing dependence, when Ca-depleting effects of narcotics are no longer evident.A rather specific model can be drawn to include narcotic analgesics, calcium levels, cyclic nucleotide metabolism as integrated biochemical events in brain that might mediate both analgesia and dependence in specific brain regions.

850 DIFFERENTIAL DEVELOPMENT OF HEMICHOLINIUM SENSITIVE AND INSENSITIVE CHOLINE KINASE ACTIVITY IN THE RAT SPINAL CORD. Alvin M. Burt. Department of Anatomy, Vanderbilt University School of Medicine, Nashville, TN 37232.

Ansell and Spanner (J. Neurochem., 22: 1153, 1974) reported that hemicholinium (HC-3) was a potent inhibitor of choline kinase (CK) activity and that the inhibition was either uncompetitive or mixed with a large uncompetitive component. Haubrich described 2 K_m values for CK (J. Neurochem. 21: 315, 1973). Both studies suggest the possibility of more than one form of CK.

In the present study the development of CK activity (total as well as HC-3 sensitive and insensitive activities) was studied in the lumbar spinal cord of rats ranging in age from 11 days' gestation (dg) through 32 days' postnatal (dp). Enzyme activity was measured with a procedure which permits the assay of CK activity in whole tissue preparations which are rich in other ATP-metabolizing enzymes (Burt and Brody, Anal. Biochem., 65: 215, 1975). A concentration of $2X10^{-5}M$ HC-3 produced the maximum inhibition of CK activity. This concentration (identical to the I_{50} of Ansell and Spanner) was used to define the HC-3 sensitive CK activity. During development, HC-3 insensitive CK activity increased from 93 μM/g prot/hr at 11 dg to a peak of 311 at 17 dg. Activity declined rapidly to 175 by 1 dp and more slowly to 112-120 by 25-30 dp. HC-3 sensitive activity increased from 53 at 14 dg to a peak of 145 at 19 dg, dropped rapidly to 59 by 4 dp and remained relatively constant throughout the remainder of development. The two ontogenetic patterns, although qualitatively similar, are 2 days out of phase. This is consistent with the concept that more than one form of the enzyme exists. (Supported by U.S.P.H.S. Grant NS-07441 and the Vanderbilt General Research Support Fund).

851 OPIATE-LIKE ACTIVITY OF NATURAL AND SYNTHETIC PEPTIDES. W. L. Byrne, E. E. Codd*, R. C. Hermann*, N. N. Santos and L. de Cato. Department of Biochemistry and Department of Pharmacology, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163.

Hughes, et al. (Nature 258 P577) reported the identification of two pentapeptides with opiate agonist activity, methionine enkephalin (MET-ENK) and leucine enkephalin (LEU-ENK), and MET-ENK corresponds to residues 61-65 in β-lipotropin. Subsequently, a series of peptides has been characterized which has properties in common with enkephalin. Some of these peptides, e.g. the C peptide of Bradbury et al., have in common an N-terminal tyrosine (position 61 of β-lipotropin), but they vary in the number of residues and appear to be more potent than MET-ENK. Using 1M acetic acid extraction of mouse brain, Amicon UM-10 filtration and gel filtration, our results indicate that approximately 20% of the enkephalin-like activity (endorphin) elutes from the column in a region which corresponds to enkephalin as judged by a ^{3}H-dihydromorphine binding assay. The binding assay plus other tests of opiate-like activity suggest multiple peptides, and the data obtained with synthetic peptides appears to modify the previously proposed role of the N-terminal tyrosine of opiate-like peptides. Based on the binding assay and the electrically stimulated ileum or vas deferens preparation, substitution at the N-terminal position of enkephalin leads to (1) loss of activity, (2) agonist activity or (3) antagonist activity. Antendorphin, ARG-MET-ENK, (personal communication from G. Ungar and synthesized by D. Sarantakis) has antagonist properties, $(Ala)_3$-LEU-ENK amide had agonist-like properties, and t-Boc-LEU-ENK was inactive.

852 EFFECTS OF AMOBARBITAL ON ADENOSINE 3':5' MONOPHOSPHATE IN RAT BRAIN. Major L. Cohn and Marthe Cohn*, Department of Anesthesiology, Magee-Womens Hospital, Univ. of Pitt. Sch. of Med., Pittsburgh, Pennsylvania 15213

The molecular events regulating narcosis are unknown. We have reported that the nucleotide cyclic AMP, dose relatedly, shortens narcosis induced by eight structurally unrelated agents. In the present study, brain cyclic AMP concentrations were sequentially measured in rats anesthetized with amobarbital. Sprague-Dawley rats (200-250 g) were injected intraperitoneally with amobarbital (80 mg/kg). The control group of rats slept 104±6.2 minutes. Following loss of the righting reflex, groups of rats were sequentially sacrificed in a high-density microwave oven at timed intervals of 0, 5, 10, 20, 25, 75, and 100 minutes. Brains were sectioned into right and left cortex, right and left caudate nucleus and hypothalamus, homogenized and extracted with perchloric acid. Supernatant was purified on ion exchange columns and cyclic AMP measured by radioimmunoassay. Our findings showed that at the same time intervals, similar cyclic AMP concentrations were found in all the areas of the brain studied. At the 5 minute interval, cyclic AMP concentrations rose two-fold above control values; at 10 minute interval, they fell sharply to control values and remained low until the 25 minute interval when they began a steady rise reaching a seven-fold increase at the 75 minute interval (corresponding to signs of very light plane of anesthesia). At time of arousal, cyclic AMP concentrations were again close to control values. Our findings suggest that 1) amobarbital-induced respiratory depression produces initial rise. Supporting our contention is the fact that anoxia is a potent stimulator of the adenyl cyclase system; 2) cyclic AMP is involved indirectly in mechanism of narcosis but directly in mechanism of arousal. Since narcosis and arousal are tightly coupled events, it is evident that cyclic AMP is a regulator of the duration of narcosis.

853 THE EFFECT OF β-BUNGAROTOXIN ON ACETYLCHOLINE RELEASE FROM BRAIN SYNAPTOSOMES. Jack R. Cooper and Indira Sen. Dept. Pharmacol., Yale Univ. Sch. Med., New Haven, CT. 06510.

The neurochemical activity of β-bungarotoxin was investigated using a synaptosomal preparation of rat cerebral cortices. In preparations preincubated with [^{3}H]choline in order to label acetylcholine the toxin caused a rapid release of the transmitter which was calcium and sodium dependent but only little affected by a depolarizing concentration of potassium. β-Bungarotoxin was also shown to be a potent inhibitor of the high affinity transport system for choline, producing 50% inhibition at a concentration of 5×10^{-8} M. These findings explain the observed electrophysiological effects of the toxin. Electron microscopy revealed no discernable effect of β-bungarotoxin at a concentration of 10^{-7} M on either synaptic vesicles or mitochondria. Neither the release of transmitter nor inhibition of choline uptake by the toxin was affected by the presence of an inhibitor of phospholipase activity.

854 LIPID COMPOSITION OF RAT CNS MYELIN-FREE AXONS DURING DEVELOPMENT. Carson J. Cornbrooks* and George H. DeVries. Dept. of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298 U.S.A.

Myelin-free axons were isolated from rat CNS during and after the period of active myelinogenesis by a modification of our procedure (J. Neurochem. 26,725. 1976). Modifications included the use of higher molarity sucrose used for floatation of myelinated axons and the use of an EGTA solution for stripping of myelin. The dry weight yield of myelin-free axons increased from approximately 0.2 mg per brain at 20 days to 0.6 mg per brain at 67 days probably due to more efficient isolation of myelinated axons from the more myelinated brains. The 20 day myelin-free axons were 12.5% lipid comprised of 74.0% phospholipid, 12.5% cholesterol, and 13.5% glycolipid. The phospholipid content of the myelin-free axons lipid did not vary at 27, 35, 60, and 67 days. However, the glycolipid content decreased to 8.2% at 35 days then increased to 14.4% at 67 days with concomitant changes in the cholesterol levels. This developmental pattern of glycolipid content does not coincide with that of rat CNS myelin glycolipid which shows a constant increase throughout this developmental period (J. Neurochem., 21, 759, 1973). On the basis of this developmental pattern and the previously determined lipid composition of adult rat CNS myelin-free axons, we conclude that the glycolipid of the myelin-free axons shows a developmental variation which does not correlate in a positive way with that of CNS myelin.
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855 NERVE FIBERS IN SURVIVING MIDBRAIN SECTIONS EXHIBITING A BLUE FLUOROCHROME UNDER U.V. LASER EXCITATION. J. T. Cummins and D. Shoemaker*, Addiction Research Laboratory V.A. Hospital, Sepulveda, CA. 91343 and the Dept. of Pharmacol. and Exptl. Therap. Univ. of Calif., Irvine.

Nerve fibers with an intense blue fluorochrome were observed when isolated coronal rat midbrain sections were illuminated at 325 nm with a He-Cd laser. The 0.3 mm sections were maintained at 37°C in vitro with oxygenated Krebs-Ringer in a perfusion chamber with a quartz window, and the fluorescence measured by a monochrometer and photoncounting system. Large, blue fluorescent fiber tracts were observed in the cerebral aqueduct (1μ) and the periventricular nucleus (0.5μ) and small fluorescent fibers (0.05-0.1μ) were infrequently observed in other brain regions. The emission peaks of the "periventricular fiber" (PF) were at 460 nm, 600 nm and 750 nm. The PF was observed to undergo movement which may be due to axonal transport. The intensity of the blue fluorescence of the PF, as measured by photon counts, was abated by the administration in vivo of chlorpromazine or amytal but not reserpine. Perfusion of the section with formaldehyde or glyoxylic acid enhanced the fluorescence of the PF. The fluorochrome remains unidentified but may be a metabolite of a biogenic amine. The U.V. laser-microfluorimeter with monochrometer and photon counting system should provide a useful tool to study metabolism of surviving brain nuclei and nerve tracts.

856 TRYPTOPHAN TRANSPORT IN MOUSE BRAIN SYNAPTOSOMES: HIGH AFFINITY UPTAKE OR HOMOEXCHANGE? J.A. Diez* and G.K. Summer* (SPON: P. Morell). Dept. of Biochem., Sch. Med., Univ. of North Carolina, Chapel Hill, NC 27514.

Kinetic studies of the active transport of tryptophan (trp) across the neuronal membrane have suggested that the amino acid is taken up by both low and high affinity transport systems. Since Levi and Raiteri have recently reported that the apparent high affinity uptake of GABA may be an artifact of homoexchange between endogenous and exogenous pools, we sought to determine whether this phenomenon might also occur with trp transport. After prelabelling synaptosomes (P_2) with 33 uM trp, we have found a stimulated-efflux of trp during a subsequent 1.0 min incubation with 10-50 uM trp. This exchange was temperature and concentration dependent. However, when trp was measured fluorometrically, we also found a net accumulation of trp, despite the occurrence of homoexchange. When the ability of 18 other amino acids or trp metabolites to inhibit isotopic trp uptake or to stimulate its efflux was studied, we found that these two effects were highly correlated. This suggests that high affinity uptake and exchange of trp are mediated by similar or identical carriers with varying affinity for other amino acids. Utilizing a gas chromatographic method for simultaneous analysis of 15 amino acids, we will report results of other studies on the stoichiometric relationships between high affinity uptake, exchange, and diffusion. We conclude from these studies that uptake and efflux of trp are coupled with bi-directional transport of several other amino acids. The apparent net uptake of trp in the fluorometric experiment (above) could therefore have resulted from exchange of exogenous trp with several other endogenous amino acids. The net uptake of a single amino acid cannot be studied without attention to the transport of other amino acids in the same transport class.

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857 INNER-EAR CARBONIC ANHYDRASE: DISTRIBUTION, PURIFICATION, AND PROPERTIES. Dennis G. Drescher* (SPON: J. Fex). LNO, NINCDS, NIH, Bethesda, MD. 20014.

Carbonic anhydrase, an enzyme which catalyzes the hydration of carbon dioxide to form bicarbonate and hydrogen ions, is present in large amounts in the mammalian inner ear where it may function in the removal of carbon dioxide and the secretion of potassium into the endolymph. The highest specific activity of carbonic anhydrase is found in the spiral ligament (including the spiral prominence and part of the outer sulcus), and next highest in stria vascularis. The auditory nerve and the organ of Corti contain little enzyme. Carbonic anhydrase from the cochlear membranous lateral wall (stria vascularis, spiral ligament, spiral prominence, and part of outer sulcus), purified to homogeneity by gel-filtration and ion-exchange chromatography, was found to be of the high-specific-activity or C type. The enzyme has a molecular weight of about 30,000 daltons and is half-maximally inhibited by 10^{-8} molar acetazolamide. Cochlear carbonic anhydrase fluoresces strongly when bound to 5-dimethylaminonaphthalene-1-sulfonamide, and can thus be identified on polyacrylamide gels after electrophoresis of cochlear homogenates.

858 TYPES A AND B MONOAMINE OXIDASE IN BRAIN AND OTHER TISSUES OF THE HAMSTER. D.J. Edwards* and C.W. Malsbury. Western Psychiatric Inst. & Clinic, Dept. Psychiatry, Univ. Pittsburgh, Sch. Med., Pittsburgh, Pa. 15261

Two forms of monoamine oxidase (MAO) are differentiated on the basis of their substrate specificity and their sensitivity to inhibition by certain drugs. Type A MAO preferentially uses serotonin (Ser) and norepinephrine as substrates and is highly sensitive to inhibition by low concentrations of clorgyline. Type B MAO preferentially uses phenylethylamine (PE) as substrate and is sensitive to inhibition by low concentrations of deprenyl but not clorgyline. In the present study, we found that MAO-A (measured with Ser as substrate) and MAO-B activities (measured with PE as substrate) in the hamster brain were only 41% and 6%, respectively, of the corresponding activities in the rat (expressed as enzymatic activity/g tissue). Since the inhibition by deprenyl when PE was used as substrate (I_{50} = 8 x 10^{-9} M) and when Ser was used as substrate (I_{50} = 3 x 10^{-6} M) was similar to that observed in rat tissues, this indicated that the small amount of PE deamination in hamster brain was due to MAO-B activity rather than to a nonspecific action of MAO. MAO-B activity was also found in the following tissues in order of increasing activity: kidney<brain<spleen<heart<lung<liver. The ratio of A:B activities was found to have a 25-fold variation among these tissues, being greatest in brain (25.2) and least in liver (1.03). Since previous reports have shown that gonadal steroids have an effect on MAO activity, we are currently investigating this possibility in the female hamster. MAO activities from brain regions containing estradiol concentrating neurons are compared to activities from other brain regions to determine whether estradiol has a differential effect on MAO-A or B and further, whether such effects are specific to those areas containing hormone target sites.

859 OPPOSING EFFECTS OF ACUTE AND CHRONIC CHLORPROMAZINE ADMINISTRATION ON THE PHOSPHORYLATION OF SPECIFIC PROTEINS IN MEMBRANES FROM RAT NEOSTRIATUM. Yigal H. Ehrlich, Thomas Gilfoil*, and Eric G. Brunngraber. Dept. Psychiat., Mo. Instit. Psychiat. Univ. Mo.-Columbia, St. Louis, Mo. 63139.

Acute and chronic treatments with neuroleptics induce opposing behavioral and physiological effects associated with altered synaptic activity. The involvement of cyclic AMP-regulated phosphorylation of membrane-bound proteins in mechanisms which underlie these alterations was investigated. Rats were isolated in individual cages for two weeks prior to drug treatment, then injected with 5 mg/kg/day chlorpromazine (CPZ) or saline and sacrificed 24 hrs after the last injection. Membrane fractions were prepared and assayed in vitro for endogenous protein phosphorylation using gamma-^{32}P-ATP (Ehrlich & Routtenberg, FEBS Lett. 45,237,1974). Compared to controls injected with saline for 21 days, protein phosphorylation was decreased in rats injected once with CPZ (after 20 days of saline), and increased in rats injected with CPZ during the last 11 days of treatment or during the entire 21 day period. The endogenously phosphorylated proteins were resolved by SDS-gel electrophoresis. The decreased phosphorylation observed after the acute treatment and the increase following the chronic treatments were associated with the specific bands designated as D-1, F, and H-1. Kinetic analysis revealed that the changes were due to alterations in the time-course of the cyclic AMP-stimulated phosphorylation of the specific proteins involved. 5 and 0.5 uM CPZ in vitro had no effects on phosphorylation of controls. The study indicates that long lasting changes in phosphorylation mechanisms are induced by CPZ in the brain in vivo which can be detected by phosphorylation assays in vitro.

860 SOCIAL SETTING: INFLUENCE ON THE INDUCTION OF BRAIN cAMP IN RESPONSE TO ELECTRIC SHOCK IN THE RAT. B. Eichelman, E. Orenberg*, E. Seagraves*, and J. Barchas. Dept. Psychiat., Sch. Med., Stanford, U., Stanford, CA. 94305.

Rats subjected to electric footshock display two disparate behaviors, escape or aggressive attack, depending on whether the rat is shocked alone or with a conspecific. Previous work suggests a correlation between aggression and cAMP (Orenberg et al., 1975). This study examined the levels of whole brain cAMP in 6 groups (N=7-8) of male Sprague-Dawley rats under the following conditions: 1) control, without shock or injection; 2) saline-injected control group; 3) shock-alone group (Escape) group; 4) shock-paired (Fighting) group; 5) caffeine-treated group, unshocked; and 6) caffeine-treated group, shocked when paired (Caffeine-Fighting). Rats were tested and/or injected for 3 consecutive days. A 2 mA shock of 0.4 s duration cycled every 7.5 s for 50 footshocks was administered in a "Plexiglas" enclosure. The saline-injected group received 2 cc ip of 0.9% saline. The caffeine-injected groups received ip 100 mg/kg of caffeine, given as the citrate, in 2 cc. The Caffeine-Fighting group received shock approximately 30-60 min after injection. On the third day, within 2-5 min after testing, each rat was killed by microwave irradiation to the head. The brains were removed, frozen, and later assayed for cAMP according to a modification of the method of Tovey et al. (1974). There was approximately a 100% increase in brain cAMP following exposure to the Fighting or Caffeine-Fighting paradigms compared to uninjected or injected controls ($p<.01$). There was a slight increase ($p \leq .05$) of cAMP in the Escape group over controls. However, the Fighting paradigm induced significantly higher levels of cAMP than the Escape ($p<.02$). This effect was not altered by caffeine pre-treatment, nor did caffeine by itself in this study significantly elevate brain cAMP.

861 EFFECT OF BROMOCRIPTINE ON THE LEVODOPA/CARBIDOPA-INDUCED CHANGES IN SEROTONIN METABOLISM.
Stanley Fahn and Stuart R. Snider*. Dept. Neurol, Columbia Coll. Phys. & Surg., New York, N.Y. 10032.

The mental side effects of levodopa or levodopa + carbidopa therapy in parkinsonism have been attributed to a depression of brain serotonin (5-HT) metabolism. l-Tryptophan administration may reverse these biochemical abnormalities (Fahn et al., Neurology 25: 861, 1975) and possibly counteract the mental side-effects as well.

Recently, bromocriptine (Br-C), an ergot alkaloid with antiparkinson action, was shown in our laboratory to increase the level of brain 5-HT in rats (Snider et al., Neurosci. Lett. 1: 237, 1975). This suggested that Br-C might also reverse the depression in 5-HT metabolism caused by levodopa. To test this, we gave groups of 10-16 rats levodopa, 250 mg/kg, with or without carbidopa, 25 mg/kg, intraperitoneally. One half of the rats in each group were pretreated 2 h before with Br-C, 5 mg/kg. Rats were killed 2 h after levodopa administration and whole brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were measured.

Brain 5-HT was reduced 26% in animals receiving levodopa and 40% in those receiving levodopa + carbidopa. 5-HIAA was also reduced. In contrast, animals pretreated with Br-C had significantly higher levels of 5-HT and 5-HIAA (32-62% above comparable groups without Br-C) and concentrations of both compounds were close to or above normal control values. These results suggest that the addition of Br-C to the therapeutic regimen of levodopa-treated patients could result in both further reduction of the motor deficit and a decrease in those side effects of levodopa which are due to 5-HT depletion.

862 MICROFRACTIONATION OF AXONAL PROTEINS SYNTHESIZED IN AXONS OF THE RABBIT HYPOGLOSSAL NERVE IN VITRO. Robert D. Frankel* and Edward Koenig. Dept. of Physiology, SUNY at Buffalo, Buffalo, N.Y. 14214.

Previous studies have demonstrated that amino acids are incorporated into axonal protein in vitro, and that this uptake is inhibited by cycloheximide but not by chloramphenicol. Myelin-free axon samples were isolated from hypoglossal nerve (rabbit) after a 3-hour incubation period in MEM containing ^{3}H-leucine in vitro. The axon microsamples were completely solubilized by a buffered solution containing 1% SDS and 0.05 M dithiothreitol and microfractionated in a polyacrylamide-agarose microgel strip system developed in this laboratory. The dried strips were subjected to autoradiography. Developed autoradiograms revealed three labelled components having nominal molecular weights of 13,000, 50,000 and 76,000. If the in vitro synthesis of only three components is a reliable indication of what is synthesized normally in the axon, it becomes readily apparent that the requirements for a protein synthesizing machinery in the axon is very modest. This could account for the apparent lack of ribosomes.

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863 GLYCOPROTEIN: GALACTOSYL TRANSFERASE IN SYNAPTIC JUNCTION COMPLEXES ISOLATED FROM RAT FOREBRAIN. J. F. Goodrum*, H. B. Bosmann* and R. Tanaka* (SPON: G. J. Thomas). Center for Brain Research and Dept. of Pharmacol., Univ. of Rochester Med. School, Rochester NY 14642

It has been postulated that surface membrane glycosyl transferases and their glycosyl acceptors may act at the synaptic junction region to regulate function in analogy to their apparent regulatory role in cell-cell interactions in other systems. A highly purified fraction of synaptic junction complexes (SJC) has been used to investigate this possibility. A UDP-galactose: glycoprotein galactosyl transferase has been characterized in the synaptosomal plasma membrane fraction (SPM) and its distribution determined in the junctional (SJC) and non-junctional (NJC) portions. Both portions demonstrate galactosyl transferase activity with and without exogenously added fetuin minus sialic acid, galactose as shown in the following table, fetuin minus sialic acid, galactose at saturating conditions:

FRACTION	SPECIFIC ACTIVITY (cpm/mg protein/hr ± S.E.)				
	0 time	0°C	boiled enzyme	+ acceptor	- acceptor
SPM	252 ± 24	312 ± 31	276	3297 ± 330	2084 ± 300
NJC	281 ± 10	356 ± 12	320	4740 ± 757	1782 ± 171
SJC	476 ± 15	529 ± 20	503	2430 ± 359	2649 ± 54

These data suggest that while the NJC fraction contains the largest amount of enzyme it is the SJC fraction which contains the most native acceptor for this system. Thus these data prove that an ectoenzyme system does exist at the synaptic junction region. The role this system may play in regulating synaptic activity or cell recognition during synaptogenesis has yet to be elucidated. (Supported by USPHS grants MH 05310 and NS 11951).

864 CHANGES IN MITOCHONDRIAL RESPIRATION WITH ALTERED BRAIN PO_2. G. Haugen* J. La Manna and F. Jobsis AUTHOR: C. Austin) Section of Neurological Surgery, Loma Linda University, Loma Linda, CA and Department of Physiology and Pharmacology, Duke University, Durham, North Carolina.

Mitochondrial respiration was monitored in vivo in a series of cats and also in neurosurgical patients undergoing microanastomosis for brain ischemia. In both cat and man, anesthesia consisted of N_2O:O_2 in a mixture of 2:1, plus the use of muscle relaxants. Blood pressure and $aPCO_2$ were maintained constant. Relative cortical PO_2 was monitored polarographically with a 25 micron platinum, teflon coated, electrode inserted into the cortex. Mitochondrial respiration was monitored by measuring the relative amounts of reduced cyt a (+a3) from the cortex using the differential dual wave length spectrophotometer technique of Jobsis. Arterial PO_2(aPO_2) was changed by varying the FiO_2 of inspired gas. In the human cases, relative brain PO_2 usually varied with changes in aPO_2 up to an FiO_2 of 60%. Similarily, there was increase in oxidation of cyt a as the brain PO_2 increased. In the majority of cats, as the FiO_2 was raised, relative brain PO_2 and oxidized cyt a both increased. The results are consistent with an interpretation of increased mitochondrial oxygen utilization with increased brain PO_2 in the in vivo cortex of cat and man anesthetized with N_2O and O_2.

865 CHARACTERISTICS OF RECEPTORS MEDIATING STIMULATION OF RAT PINEAL PHOSPHOLIPID METABOLISM BY NOREPINEPHRINE (NE) AND DOPAMINE (DA). George Hauser and M. S. Nijjar*. McLean Hospital, Harvard Med. Sch., Belmont, MA 02178.

Catecholamines greatly increase $^{32}P_i$ incorporation into phosphatidylglycerol, phosphatidic acid and, primarily, phosphatidylinositol in rat pineal gland in vitro (JBC (1973) 248, 3615). The action of NE can be blocked by phenoxybenzamine or phentolamine, but not by sotalol, and hence appears to be mediated by α-adrenergic receptors (Nature (1974) 252, 482). In order to define more precisely the mode of action of DA and to compare it with that of NE, we have utilized the DA-receptor blocking agent haloperidol (HP). Individual intact pineal glands were incubated in 100 µl of Puck's N-16 medium with $^{32}P_i$ for 60 min before extraction and separation of the lipids. HP blocked the effect of 30 µM DA partly at 1 µM and completely at 10 µM whereas the effect of 30 µM NE was not diminished by 30 µM HP. At higher concentration HP also reduced the NE effect. When glands were preincubated for 15 min with 5 µM HP, NE still gave considerable stimulation of phospholipid metabolism but DA did not. The action of the α-agonist clonidine is less pronounced than that of NE but is unaffected by preincubation of the glands with HP. It may thus be more specific than NE. In contrast to observations in other systems the action of DA was not mimicked by apomorphine at any concentration. However, DA does not act by first being converted to NE since DA β-hydroxylase inhibitors did not prevent the DA-induced stimulation. The findings can be explained by the existence of separate receptor sites for the two catecholamine agonists in the pineal gland. However NE may in part be able to act through the DA receptor as suggested by the HP blockade of the NE-induced stimulation. Phenoxybenzamine and phentolamine which abolish both the NE and DA effects do not seem to be uniquely specific for α-adrenergic receptors. (Supported by USPHS grant NS06399 from NIH.)

866 THE EFFECT OF MATERNAL ALCOHOL CONSUMPTION ON CNS MYELINATION IN THE DEVELOPING RAT.

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Pregnant Sprague-Dawley rats were pair-fed, using either the control or 6.6% (v/v) ethanol liquid diets, described by Lieber and DeCarli (J. Med. Prim. 3:153, 1974). At birth, litters were adjusted to 10 pups and the ethanol content of the alcohol diet was halved. Two days after delivery, all rats were placed on chow, ad lib. Pups were weighed regularly. At intervals between 17 and 53 days of age, pups were injected intracerebrally with 150 µCi (^{3}H)leucine and 20 µCi (^{14}C)glucose. Rats were sacrificed 18 h after injection, and myelin was isolated (JNC 21:749,1973) and subfractionated into light, medium and heavy myelin (BBA 329:305,1973). Myelin subfractions were analyzed for protein content and distribution of radioactivity.

Offspring of alcohol-fed mothers (alcohol pups) had lower brain and body weights and a higher "infant mortality" rate than control rats. Although we did not observe a deficit of CNS myelin in alcohol pups, we did observe that their myelin had an abnormal composition. Alcohol pups had a significantly greater proportion of heavy myelin and significantly greater synthesis of the heavy fraction than did control rats. These findings suggest that the myelin of alcohol pups is abnormal and typical of more immature animals. Supported in part by USPHS AA00266.

867 PRODUCTION OF ANTIBODY TO ENCEPHALITOGENIC PEPTIDE IN RABBITS AND CHICKENS. Ted C. Hung* and Helene C. Rauch (SPON: J.A. Benjamins). Dept. Immunol. and Microbiol., Wayne State U. Sch. Med., Detroit, MI 48201.

Myelin basic protein (MBP) induces experimental allergic encephalomyelitis (EAE) in laboratory animals. The synthetic tryptophan containing nonapeptide representing residues 114-122 is encephalitogenic for guinea pigs; the tryptophan appears to be essential for biologic activity. Tryptophan is not required for encephalitogenic activity in species other than guinea pig. We have immunized rabbits and chickens with this peptide conjugated with bovine serum albumin (BSA) or polymerized alanine and lysine (poly AL) by the use of carbodiimide. Repeated injections were done with conjugates emulsified in incomplete and complete Freund's adjuvant over a period of months. Sera were assayed by micro-immunodiffusion employing 0.8% agarose in 0.1 M sodium acetate, pH 7.4. Cross reactivities were observed in some sera with MBP and peptide conjugated with unrelated carrier which suggested the presence of anti-peptide antibodies in these animals. Specific anti-peptide antibody was demonstrated by affinity column chromatographic separation. Antibodies also reacted with MBP treated with 2-hydroxy-5-nitrobenzyl bromide which modifies the tryptophan residue suggesting that tryptophan is not involved in the antigenic determinant.

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868 ENZYMATIC AND IMMUNOLOGICAL DETERMINATIONS OF A NEURON SPECIFIC PROTEIN (NSP-R). Angelica Keller* and Claire Zomzely-Neurath. Dept. Biochem., Roche Inst. Molec. Biol., Nutley, N.J. 07110.

The neuron specific protein, isolated from rat brain (NSP-R, homologous to the beef 14-3-2 protein) has an enolase enzymatic activity. We now report an enzymatic assay for total and brain specific enolase activities in rat brain. Brain specific enolase activities (units/mg protein) were determined at 22°C in 4 brain regions: cortex, 0.47; medulla, 0.43; brain stem, 0.70; cerebellum, 0.57; representing 60-67 per cent of the total enolase activity. No marked difference was observed between brain regions, confirming the results obtained when measuring NSP-R levels by radioimmunoassay. Furthermore, total enolase activity was measured in the rat C6 glioma cell line (0.80 units/mg protein) and no brain specific enolase activity was detected. NSP-R is absent in this cell line as measured by radioimmunoassay whereas S100, a glial protein, is present. Significant changes in the NSP-R levels have previously been observed during "T"-maze performance tasks with food reinforcements as measured by radioimmunoassay. Data will be presented on the relationship of the increased NSP-R levels and brain specific enolase activity. These results should contribute to an elucidation of the physiological role of this neuron specific isozyme.

869 PRELIMINARY ESTIMATES OF THE DOPAMINE β-HYDROXYLASE CONTENT AND ACTIVITY IN PURIFIED NORADRENERGIC VESICLES. Richard L. Klein, Donny F. Kirksey*, Robert A. Rush* and Menek Goldstein. Dept. Pharmacol. Toxicol., Univ. Miss. Med. Ctr., Jackson, MS 39216; Roche Inst. Mol. Biol., N.J.; Dept. Neurochem., NYU Med. Ctr., NY.

The dopamine β-hydroxylase (DβH) content and activity of large dense core noradrenergic vesicles purified from bovine splenic nerve by the sucrose-D_2O density gradient method were determined using two assay procedures: enzymic activity expressed in Units/mg protein and homospecific activity based on radioimmunoassay expressed in Units/mg DβH antigen, and compared with concanavalin A-Sepharose purified enzyme. Two-thirds of the total enzyme activity was latent in these vesicles, even after various treatments designed to compromise vesicle integrity. DβH activity was completely unmasked by brief treatment with Triton X-100; activity increased from 0.20 to 0.64 Units/mg vesicle protein. Calculations based on both assay procedures suggested that an average of ∿ 7% (range 3 - 15%) of the total vesicle protein was DβH and the average vesicle contained ∿ 4 molecules of enzyme (range 2 - 9 molecules). Estimated homospecific activities indicated an average 25 to 50% (range 18 - 72%) of the vesicle enzyme was inactive in various samples using two different antibodies. The vesicle can synthesize up to ∿ 30 molecules of noradrenaline/sec/molecule DβH at near optimal substrate concentration. The assumptions used in the various calculations were critically analyzed and, based on the methods employed, it is tentatively considered to be unlikely that there could be more than 5 to 12 molecules of DβH per vesicle. It is suggested that circulating DβH originates primarily, if not exclusively, from the large dense core noradrenergic vesicle type.

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870 CHANGES IN ENERGY METABOLITES DURING AND AFTER BILATERAL ISCHEMIA OF THE CEREBRAL CORTEX OF THE GERBIL. M. Kobayashi*, W. D. Lust, J. V. Passonneau and I. Klatzo. NIH, Bethesda, MD. 20014.

Occlusion of both common carotid arteries of the gerbil results consistently in bilateral ischemia of the cerebral cortex permitting investigation of the effects of short-term ischemia on cerebral energy reserves Biochemical evidence in the present study confirms cerebral ischemia in all gerbils subjected to bilateral carotid occlusion. Previous studies in our laboratory indicated that the changes in metabolites during recirculation may reflect the intensity of the ischemic insult more accurately than alterations which occur during ischemia. To pursue these observations the levels of energy reserves were measured at 1 m, 5 m, 30 m, 1 h and 6 h of recirculation following 1, 5, 20, 30, and 60 m of bilateral ischemia. Brain glucose decreased progressively during ischemia, but increased 3-fold over controls after 30 m of recirculation. Glycogen levels decreased rapidly in ischemic brain, slowly increased during recirculation and were 2-fold greater than control after 6 h of recirculation. Lactate increased 11-fold during ischemia; the time required to return to normal varied directly with the duration of the ischemic insult. ATP and P-creatine decreased to 20% of normal after all periods of ischemia. ATP levels were restored to 80% of control after 30 m of recirculation, except in 1 h ischemic animals which were 26% of control. In contrast, P-creatine increased to greater than control following 30 m of recirculation in all groups. The rate of recovery of ATP and P-creatine was dependent on the duration of ischemia as observed with lactate. The total adenylate pool decreased during ischemia to 45% of control and was not fully restored after 6 h of recirculation. These results confirm our earlier observations that the intensity of the ischemic insult affects not only the changes during ischemia, but also the rate and extent of recovery.

871 CONCENTRATION OF THE ANTI-ENCEPHALITOGENIC SPINAL CORD PROTEIN (SCP) IN AXONS. C. F. C. MacPherson and A. C. Wallace*. Departments of Psychiatry and Pathology, University of Western Ontario, London, Ontario, N6G 2K3

Immunohistological studies using a rabbit anti-serum prepared with purified bovine spinal cord protein (BSCP) (J. Immun. 109:1009,1972) and fluorescein-labelled goat anti-rabbit IgG showed staining of round structures resembling nerve fibres in cross-sections of bovine and human spinal nerve roots and sural nerves. Evidence that the fluorescence was confined to axons was obtained when consecutive sections of tissues stained to reveal axis cylinders or myelin confirmed that the fluorescence was not in the myelin sheaths. Weaker staining was observed in nerve fibres in brain and in the walls of cerebral blood vessels.

BSCP is a non-encephalitogenic organ-specific antigen of mammalian nervous tissue. Pretreatment with purified BSCP protected guinea pigs from experimental allergic encephalitis (EAE) when they were immunized with bovine spinal cord or bovine myelin basic protein (MyBP). The mechanism of the protection afforded by SCP is not clear. SCP and MyBP differ with respect to their amino acid composition, molecular weight and surface charge: moreover, they yield patterns of non-identity when compared by immunodiffusion experiments. Thus the anti-encephalitogenic activity of SCP does not depend on antigenic sequences shared with MyBP but may be related to its location in the axon.

Supported by the Multiple Sclerosis Society of Canada.

872 THE STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THY-1 ISOLATED FROM MOUSE BRAIN SYNAPTIC MEMBRANES. Larry D. McClain and Ronald T. Acton. Dept. Microbiol., UAB, Birmingham, Alabama 35294

The Thy-1 alloantigen has been shown to be expressed on isolated synaptic plasma membranes and synaptic junctional complexes from mouse brain. Utilizing zonal centrifugation techniques Thy-1 has been found to be localized on a plasma membrane fraction of neuronal origin as monitored by enzyme marker activities. Further characterization of the distribution of this cell surface antigen by immunoferritin electron microscopy and immunofluorescent labelling has confirmed this localization at the synaptic region. These findings suggest that Thy-1 represents a specific marker for synaptosomes, synaptic membranes, and junctional complexes. Due to the antigenic lability of this molecule in both ionic and non-ionic detergents, selective extraction of butanol washed acetone powder preparations has been utilized to isolate Thy-1 from mouse brain. In an effort to describe the possible role of this membrane component in the establishment and/or maintainence of synaptic connections chromatographically isolated soluble fractions of Thy-1 have been introduced into cultures of dissociated embryonic mouse brain cells. Interference in the aggregation or subsequent cell type specific resorting in these cultures will confirm the efficacy of this approach in determining the precise role of Thy-1 in synaptic metabolism.

873 AMINO ACID LEVELS IN MOUSE BRAIN DETERMINED AFTER MICROWAVE FIXATION. J. L. Meyerhoff, R. H. Lenox, and N. D. Brown*. Dept. Neuroendocrinology, Div. Neuropsychiatry and Div. Biochemistry, Walter Reed Army Inst. of Research, Walter Reed Army Medical Center, Washington, D.C. 20012.

Exposure to high-intensity microwave irradiation has been utilized as a means of in-vivo enzyme inactivation prior to assay of brain levels of a number of metabolites and putative neurotransmitters. Whole brain levels following irradiation have compared favorably with values observed following rapid freezing techniques. Our laboratory has previously demonstrated the use of microwave irradiation for the determination of levels of GABA in rat brain. A number of amino acids are rapidly metabolized and are closely linked metabolically with pyruvate or Krebs cycle intermediates. Studies using liquid nitrogen immersion have reported post-mortem changes occurring in amino acid levels in rat brain. We decided to examine the effect of microwave fixation on levels of a range of amino acids in mouse brain. Swiss Webster Walter Reed strain mice weighing approximately 25 grams each were sacrificed by decapitation into liquid nitrogen, by decapitation at room temperature or by exposure to microwave irradiation at a frequency of 2450 MHZ, at 2.5 KW for 1 second. Samples were sonicated in 10% trichloroacetic acid and quantified by amino acid analyzer. The following amino acids were measured: alanine, taurine, aspartic acid, threonine, serine, GABA, glutamine, lysine, histidine, arginine, glutamic acid, glycine, valine, leucine, tyrosine and phenylalanine. There was a very marked post-mortem increase in levels of alanine in brains of mice decapitated at room temperature. The significant post-mortem elevation in GABA as well as absence of change in glutamate previously demonstrated in rats was also observed in this study. Glycine levels were not affected by method of sacrifice. It would appear that the microwave technique has applicability to the study of amino acids in brain.

874 A BRAIN PROTEIN ASSOCIATED WITH THE "SCHIZOPHRENIC SPECTRUM" OF MENTAL DISORDERS. Gary D. Miner*, William A. Rush*, Stephen J. Mayor, and Leonard L. Heston*. Psychiatry Research Unit, University of Minnesota, Minneapolis, MN. 55455, and Department of Physiology, Medical College of Ohio, Toledo, OHIO 43614.

Electrophoretic brain protein polymorphism studies in humans revealed a protein (called "Sc-P") missing in several cases of schizophrenia (p=0.009), schizoids blindly diagnosed (p=0.02), and demented conditions with schizophrenic-like symptoms (p=0.02). No association between the presence or absence of the protein and age, sex, drug history, terminal illness, or institution having custody was discovered. Computerized-densitometry developed by Dr. Stephen J. Mayor, coupled to a cluster analysis statistical design, further illustrated this protein abnormality and indicates that at least two other proteins may be implicated with the "schizophrenic spectrum" from our pathological samples. Since proteins are the products of genes, it is possible that the "Sc-P" protein is related to the genetic predisposition existent in schizophrenia, or it may be the result of the external or internal environmental stresses present in this disease.

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875 TURNOVER OF ELECTROPHORETICALLY SEPARATED FUCOSYL GLYCOPROTEINS IN THE RAT NEOSTRIATUM. David Morgan* and Aryeh Routtenberg, Cresap Neurosci. Laboratory, Northwestern University, Evanston, Illinois, 60201.

The time course of fucose incorporation into glycoproteins of the rat neostriatum was examined using intracranial injections. The first experiment measured the diffusion characteristics of the injected material. 25 μC of L-[6-^{3}H] fucose were injected in 1 μl volume over 4 min. The animals were sacrificed 15 min, 30 min, 1 h, 3 h, 6 h and 12 h after the injection. The neostriatum and the overlying cerebral cortex, both ipsilateral and contralateral to the site of injection, were dissected and homogenized in 10% TCA. Although little fucose was incorporated into glycoproteins initially, the relative specific activity increased from 0.19 at 1 h to 2.8 at 6 h and 3.8 at 12 h. Labeling of the ipsilateral cerebrum was weaker and delayed with respect to the neostriatum, and labeling was minimal in the contralateral cortex and neostriatum.

The second experiment examined the time course of fucose incorporation into neostriatal glycoprotein separated by 7-18% linear gradient polyacrylamide electrophoresis. At 2.5, 3, 4, 5, 8 or 26 hours following injections, crude synaptosomal fractions from the injected neostriatum were prepared and dissolved in 3% SDS, 2% 2-mercaptoethanol and 5 mM Tris-acetate (pH=8.0). Samples were electrophoresed for 25 hours on 11 cm slab gels, which were then sliced and counted. At all time points examined, a nearest neighbors summation analysis revealed 10 distinct glycoprotein peaks ranging from 100,000 to 20,000 daltons. However, the relative sizes of these peaks changed over time suggesting separate turnover rates. The effect of passive avoidance training on the incorporation of ^{3}H-fucose into these peak regions was examined. Supported by the Alfred P. Sloan Foundation, NS 10768, MH 25281 and BMS 19481 grants to A. R.

876 INCREASED RATE OF DISAPPEARANCE OF SERUM PROBENECID: A PROBLEM IN MEASURING SEROTONIN TURNOVER IN BARBITAL DEPENDENT RATS. W.W. Morgan. Dept. Anat., Univ. of Texas Health Sci. Ctr. at San Antonio, San Antonio, TX. 78284

Adult male Sprague-Dawley rats were made barbiturate dependent by the administration of increasing concentrations of barbital in their drinking water for six weeks. The barbital rats were withdrawn from this drug for 0 or for 1 day before sacrifice. Control and barbital dependent rats were sacrificed 0, 30, 60 or 90 minutes after the intraperitioneal administration of probenecid (200mg/kg). In 0 or 1 day withdrawn barbital dependent rats the accumulation of 5-hydroxyindoleacetic acid (5-HIAA) induced by probenecid was significantly reduced in the midbrain ($p<0.05$), cerebral cortex ($p<0.005$) and medulla pons ($p<0.025$). In a repeat study blood serum was also collected for the analysis of circulating probenecid levels by gas chromatorgraphy with flame ionization detection. The serum levels of probenecid were significantly decreased ($p<0.01$) in barbital dependent versus control rats by 60 and again at 90 minutes following probenecid administration. A metabolite of probenecid was significantly elevated ($p<0.001$) in barbital dependent rats compared to controls by 30 minutes after probenecid administration. These results suggest that the decreased accumulation of 5-HIAA in brain areas of barbital dependent rats may be the result of an increased metabolism of probenecid rather than the result of a true decrease in serotonin turnover. (Supported by NIDA Grant # 5 R01-DA0075 and Research Scientist Development Award 1 K02 MH 0028 from the National Institute of Mental Health)

877 IN VITRO DEHYDROXYLATION OF p-TYRAMINE BY RABBIT BRAIN PREPARATIONS. Aron D. Mosnaim, Stephan R. Cann*, Dorrel L. Edstrand*, Marion E. Wolf*. Dept. Pharm., Chicago Med. Sch./Univ. Health Sci., Dept. Psych., Rush Presby.-St. Luke's Med. Center, Chicago, Ill. 60612

Ring dehydroxhylation of biogenic amines has been reported by a number of investigators. We have demonstrated the in vivo brain interconversion of p-tyramine (TRM) and 2-phenylethylamine (PEA) (Silkaitis and Mosnaim, Brain Res. 1976, in press). In this study we examine the possible ring dehydroxhylation of TRM to PEA in vitro. Rabbit brains were homogenized in hypotonic phosphate buffer (pH 7.4; 2.5% in Triton X-100), after centrifugation (10.000 g X 10 min) the supernatant was used as a crude enzyme preparation. After the addition of labeled TRM, the mixture was incubated (37°C) and samples were removed at different time intervals. PEA was extracted after the method of Mosnaim and Inwang (Anal. Biochem. 1974), and estimated by liquid scintillation counting. ^{3}H-PEA and ^{3}H-TRM were used, respectively, as internal standard for recovery and to verify the specificity of the extraction procedure. Further confirmation of the identity of the compound was obtained by TLC followed by TLC radioscanning analysis. The effects of pre-incubation with MAO inhibitors, varying substrate and crude enzyme concentrations, different incubation times and boiling of the crude enzyme preparation were evaluated. Whereas there was no PEA recovered either when using boiled enzyme preparations or in the absence of MAO inhibitor, counts corresponding to PEA were found in the presence of pargyline. The results obtained using varying substrate and enzyme concentration were consistent with an enzymatic reaction. Since it has previously been shown that there is in vivo dopamine conversion to TRM, the present results would indicate the possible existence of a brain biochemical pathway linking PEA and catecholamines. This work was supported by G.R.S.G. FR-5366 from the National Institutes of Health.

878 DIFFERENTIAL EFFECTS OF THE ACQUISITION-ENHANCING DRUG PIRACETAM ON PROLINE RELEASE FROM RAT VISUAL AND PARIETAL CORTEX IN VITRO.
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Pyrrolidon acetamide (Piracetam) enhances acquisition and enlarges cortical evoked responses. In the present work the effect of Piracetam was studied on the cortical metabolism of proline, which is an inhibitory transmitter candidate and impairs memory formation if injected intracerebrally. Slices of visual and parietal rat cortex were loaded with 0.05 μM ^{3}H-proline after pre-incubation in Krebs-Ringer bicarbonate (KRB) medium. Slices were superfused and 2-min samples were collected. After 40 min of spontaneous efflux, slices were exposed intermittently to either control medium or medium containing 10 mM Piracetam or 50 mM K^{+}. The efflux of ^{3}H-proline from visual cortex was depressed reversibly by Piracetam, whereas that from parietal cortex was enhanced reversibly. Piracetam influenced the uptake of ^{3}H-proline in neither cortical part. Exposure to 50 mM K^{+} enhanced the efflux from visual cortex more than that from parietal cortex. Pre-incubation with 50 mM K^{+} increased the uptake of ^{3}H-proline in visual cortex but had no effect on that in parietal cortex. The regional variation in the effect of Piracetam on cortical proline release may be due to regional variations in proline metabolism or to regional differences in the influence of neighbouring tissue elements on proline-releasing cells.
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879 BRAIN NUCLEAR PROTEIN KINASES: EVIDENCE FOR THEIR HETEROGENEITY AND DIFFERENTIAL RESPONSE TO CYCLIC NUCLEOTIDES AND S-100.
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Non-histone chromosomal protein preparations, isolated from rat brain nuclei, contain endogenous protein kinase activity which catalyzes phosphorylation of endogenous proteins in vitro in the presence of nucleoside triphosphates. Among the three γ-labelled nucleoside triphosphates tested, the efficiency of phosphate donors was found to be as follows: ATP>GTP>UTP. Protein kinase activity was measured in the presence of Mg^{++} and (γ-P^{32}) ATP by millipore filter assay. The nuclear protein kinases could be separated by phosphocellulose chromatography into at least eight distinct enzyme fractions. Five of these were tested for the effect on phosphate incorporation of S-100, casein, a heat-treated non-histone protein fraction, cyclic AMP and cyclic GMP. With S-100, casein and heat-treated non-histone protein as substrates, differential effects were observed with different kinase fractions: casein showed increased incorporation with 4 of the 5 fractions; whereas S-100 and heat-treated non-histone protein showed increased incorporation with some fractions, decreased incorporation with others. Furthermore cyclic AMP and cyclic GMP exhibited differences in their stimulatory or inhibitory effects on endogenous protein phosphorylation of the different protein kinase fractions. With one fraction, cyclic AMP stimulated activity whereas cyclic GMP inhibited activity. These results show that nuclear protein kinases associated with non-histone chromosomal proteins of brain can be separated into a number of enzyme fractions with differential response to S-100 and cyclic nucleotides. These kinases may play an important role in tissue-specific regulation of RNA synthesis and chromatin function.

880 CARRIER-MEDIATED AND NON CARRIER-MEDIATED RELEASE OF ^{3}H-NOREPINEPHRINE FROM HYPOTHALAMIC SYNAPTOSOMES. M. Raiteri*, R. del Carmine*, A. Bertollini*, and G. Levi* (SPON: D. L. McIlwain). Instituto di Farmacologia, Univ. Cattolica and (G.L.) Lab. de Biologia Cellulare, CNR, Rome, Italy.

It is generally agreed that norepinephrine (NE) is transported into noradrenergic nerve terminals by a carrier-mediated mechanism present in the plasma membrane. On the other hand, both carrier-mediated and non carrier-mediated mechanisms have been proposed for the release of the amine from nerve terminals. In the present study we attempted to discriminate between carrier-mediated and non carrier-mediated release of ^{3}H-norepinephrine (^{3}H-NE) from hypothalamic synaptosomes by treating the ^{3}H-NE-prelabeled particles with 10^{-5}M desmethylimipramine (DMI), for 10 min, and then studying the release of the amine in superfusion conditions. The release of ^{3}H-NE induced by 56mM KCl, by the calcium ionophore A23187 or by the lack of K^{+} was unaffected or only slightly decreased by DMI pretreatment, suggesting the involvement of a non carrier-mediated (exocytotic?) mechanism. Also the release of ^{3}H-deaminated metabolites induced by 10^{-6}M reserpine was totally unaffected by the DMI pretreatment. On the other hand, the release induced by Na^{+}-free medium or by 2-phenylethylamine seems to be carrier-mediated since it was strongly inhibited in DMI pretreated synaptosomes.

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881 Effect of Zuclomiphene, AY9944 and Triparanol on CNS Cholesterol Formation. R. B. Ramsey and V. W. Fischer, Depts. of Neurology and Anatomy, St. Louis Univ. School of Medicine, St. Louis, MO. 63104

Developing rats were administered 5 doses of a combination of hypocholesterolemic agents, zuclomiphene (30mg/kg body wt), AY9944 (3mg/kg) and Triparanol (30mg/kg), between 4 and 20 days of age. Under these conditions, the beginning of cytoplasmic inclusion body formation was demonstrable in the CNS of 20 day old animals, but did not involve the neuronal perikarya. The brain and spinal cord of these animals were rich in 7-dehydrosterols, despite general lack of inclusion bodies. 7-Dehydrocholesterol and 7-dehydrodesmosterol accounted for approximately 50% of the total CNS endogenous sterol. In comparison to control animals, in vivo incorporation of (2-^{14}C) mevalonic acid into the brains of the treated animals showed a slight reduction of total labeled sterol, as determined by digitonide formation. ^{14}C-Cholesterol formation, estimated by the cholesterol dibromide, was markedly depressed in the test animals. Chromatographic examination of the labeled sterol fractions by means of $AgNO_3$-TLC and radioactivity monitored-GLC indicated that, unlike previous animals treated with AY9944 plus zuclomiphene or AY9944 only, there was not significant labeling of sterols with conjugated double bond systems, such as 7-dehydrocholesterol or 5α-cholesta-8,14-dien-3β-ol within the limits (5h) of the experiment. Methyl sterols, such as lanosterol, had a greater portion of the total label in the test animals than in controls. Cholesterol, lanosterol, 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol, 4α-methyl-5α-cholesta-8,24-dien-3β-ol and zymosterol were found to be appreciably labeled in the brain tissue of the drug treated animals.

882 ACETYLCHOLINE RELEASE BY THE IONOPHORE X537A (LASOLOCID). Judith A. Richter. Inst. of Psychiatric Research and Dept. of Pharmacology, Indiana University Med. Sch., Indianapolis, IN. 46202.

An in vitro superfusion system was used to examine the release of acetylcholine (ACh) by X537A and KCl from rat brain slices. Preliminary experiments showed that after prelabelling tissue with ^{3}H-choline, 1-30 uM X537A and 12.5-100 mM KCl in the superfusing medium enhanced the release of radioactivity (presumably ^{3}H-ACh) in a dose-dependent fashion. Subsequent studies on striatal slices showed that 30 uM X537A and 50 mM KCl released endogenous ACh from the tissue. Potassium-induced release was dependent on extracellular Ca++ and inhibited by pentobarbital whereas the ionophore-induced release was independent of Ca++ and not inhibited by the barbiturate. In partition experiments X537A did not transport ACh or choline into a lipid phase. The ACh release by both agents reached an early peak and then declined with continued superfusion. While the addition of choline to the superfusing medium maintained K+-stimulated release at or near peak levels, it did not affect the decline in ionophore-induced release. ACh in the tissue at the end of superfusion was lower after X537A than after KCl. X537A did not inhibit choline acetyltransferase activity measured in striatal homogenates, but it did inhibit the uptake of ^{3}H-choline by striatal synaptosomes. These results suggest that the ionophore releases ACh by a mechanism different from potassium and then subsequently inhibits ACh release, perhaps by inhibiting the uptake of precursor choline. (Supported by USPHS Grant No. DA 60796).

883 CELL SURFACE LOCALIZATION OF ONE OF THE MULTIPLE MOLECULAR FORMS OF ACETYLCHOLINESTERASE (AChE) IN CULTURED MAMMALIAN AND AVIAN NERVE CELLS. François Rieger*, Prentiss Taylor* and Lloyd A. Greene* (Spon: Morris L. Karnovsky) Dept. of Neuroscience, Children's Hosp. Med. Ctr. and Dept. of Neuropathology, Harvard Med. Sch., Boston, MA 02115.

Several recent studies have shown that mammalian and avian tissues have different molecular forms of AChE, which may be separated by sucrose gradient centrifugation. We studied the differential cellular localization of these forms with regard to previously reported cell surface and internal pools of AChE. Cultured cells were exposed to a reversible, poorly lipid soluble AChE inhibitor (BW284C51) and an irreversible, highly lipid soluble cholinesterase inhibitor (diisopropylfluorophosphate, DFP). We used primary chick embryo sympathetic neurons and a clonal cell line of hybrids between murine neuroblastoma and murine sympathetic ganglion cells known as NS-31T28. For both the 10S form of AChE in NX-31T28 and the corresponding 11S form in chick sympathetic neurons, we found a high degree of protection (75-95%) by the impermeant reversible inhibitor against irreversible inhibition by DFP. In contrast, the other molecular form already described in these cells, the 4S form in the murine cells and its corresponding chick 6.5S form, was nearly completely inhibited (only 5-15% of the control activity remained). Broken cell preparations or long exposure to the BW compound showed some similar protection against DFP inhibition of the murine 4S or the chick 6.5S forms.

Consequently, we propose that the 10S and 11S forms of AChE (in murine and chick nerve cells respectively) are preferentially localized on the external cell membrane, while the 4S and 6.5S forms are localized more internally. Our results suggest that the 10S form (or the 11S form) can be used as an external enzymatic marker for subsequent studies of regulatory mechanisms for this important functional component of the neuronal plasma membrane. Supported by funds from CNRS (France) and NIH (U.S.A.).

884 REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE LEVEL AND ELECTRORETINOGRAM. Carlos Rodríguez-Estrada
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Ottoson and Svaetichin (Acta physiol. scand. 29 Suppl. 106) have reported that anoxia abolished optic nerve action potential and electroretinogram in frog's retina. Their findings suggest that origin and transmission of nerve action potential are dependent on aerobic metabolism. This work intended to correlate respiratory chain activity and electroretinogram. Fluorometric determination of reduced nicotinamide adenine dinucleotide ($NADH_2$) level was made simultaneously with transretinal potential (ERG) in in vitro preparation of toad's retina. Each retina of a light adapted eye was placed in a chamber, which was circulated with moistened oxygen or nitrogen. Under dim light illumination a flash of white light of one second duration was applied every ten or fifteen seconds. It was found that during anoxia, the $NADH_2$ level increased and the ERG changed in amplitude. In anoxia, ERG change varied from preparation to preparation while was reversible in oxygen. In a prolonged period of anoxia the decrease of the amplitude of the ERG developed gradually until it was not observed. These results indicate that respiratory chain supplies the energy required for the retinal cells involved in the ERG, and suggest that energy supplied from other metabolic pathways are insufficient to restore the energy needed to maintain the excitation level of retina's cells.

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885 LOCAL CEREBRAL GLUCOSE UTILIZATION FOLLOWING ACUTE OR CHRONIC MORPHINE ADMINISTRATION AND WITHDRAWAL. Osamu Sakurada*, Mami Shinohara*, Werner A. Klee*, Charles Kennedy, and Louis Sokoloff. National Institute of Mental Health, Bethesda, Maryland, 20014, USA.

The pharmacological effects of morphine administration and withdrawal suggest that there may be regional specificity within the brain. The recently developed autoradiographic [^{14}C]deoxyglucose method measures the rates of local cerebral glucose utilization (LCGU), and it has proved capable of identifying local areas of altered functional activity in the brain by detection of changes in their metabolic rate. Science, 187, 850-853, 1975). This method has been applied to the pharmacological effects of morphine administration and withdrawal in the rat. The acute subcutaneous administration of 5 to 30 mg per Kg of morphine produced dose-dependent reductions in LCGU in many areas of the brain, but these were closely correlated with and probably attributable to an associated respiratory depression and increased arterial pCO_2. Chronic morphine administration achieved by the subcutaneous injection of 150 mg/Kg of morphine base in an oil emulsion 24 hours before the study resulted in significant reductions in LCGU in 15 of 30 gray structures; there were no significant effects in any of the white structures. These effects were observed in the absence of significant changes in arterial pCO_2. Naloxone (1 mg/Kg subcutaneously) reduced LCGU in a number of structures when administered in normal rats, but when given to the chronic morphine-treated animals to provoke an acute withdrawal syndrome, it reversed the reduction of LCGU in several of the structures. The reversal was particularly striking in the habenula. These results are consistent with a broad mixture of specific and non-specific effects of chronic morphine administration with reversal by naloxone only in those structures, like the habenula, which are rich in morphine receptors.

886 BRAIN NICOTINIC ACETYLCHOLINE RECEPTOR IS A GLYCOPROTEIN. Paul M. Salvaterra*, James W. Gurd and Henry R. Mahler. Chemistry Department, Indiana University, Bloomington, IN 47401 and Biochemistry Department, University of Toronto, West Hill, Ontario.

The interaction of rat brain nicotinic acetylcholine receptor (nAChR) with various plant lectins (concanavalin-A, Con A; wheat germ agglutinin, WGA; Ricinus communis, RC; fucose binding protein, FBP; soy bean agglutinin, SBA; and lentil lectin, LcH) was studied by means of ^{125}I labeled α-bungarotoxin binding. Con A has little effect on toxin binding to membrane bound nAChR (only 10-20% inhibition at 1 mg/ml). Also, none of the lectins significantly inhibit toxin binding to nAChR solubilized with Triton X-100. Affinity columns were prepared with Con A, WGA and FBP attached to Sepharose and RC attached to zirconium clad controlled pore glass. Triton extracts were chromatographed and elution of any bound nAChR was accomplished by addition of appropriate sugars to the elution buffer. Brain nAChR binds to Con A-Seph (eluted with α-methyl-D-manoside) and WGA-Seph (N-acetylglucosamine) but not to the FBP (fucose) and RC (D-galactose) resins. Parallel experiments carried out with nAChR derived from *Torpedo californica* electroplax yielded identical results. These results indicate brain nAChR is probably a glycoprotein and that lectin and toxin binding sites do not overlap. (Supported by Research Grant NS 08309. PMS supported by NIH Fellowship No. 5008)

887 RELATION OF MORPHOLOGICAL CHANGES AND RNA DIVERSITY IN CLONED MUSCLE AND NEUROBLASTOMA CELL LINES. B. K. Schrier, L. D. Grouse*, D. A. Boenning* and P. G. Nelson. Behav. Biol. Br., NICHD, NIH, Bethesda, MD. 20014.

Diversity of sequences expressed in heterogeneous nuclear RNA in muscle and neuroblastoma cell lines was measured by hybridization of the RNA in excess with unique sequences of homologous DNA (uDNA). Subclones of a cloned rat muscle cell line (L-8), one of which fused to form myotubes and the other remained unfused and epithelioid, both showed transcriptional diversity of 3.1% of the uDNA (6.2% of the sense strand of uDNA, approx. 1.0% of total DNA). Re-cycling of reacted and unreacted uDNA showed greater than 90% identity of expressed sequences in both clones. Marked morphological differences in the subclones were related to minor differences in RNA diversity. A subclone (S20-F) of the cholinergic mouse neuroblastoma clone NS-20 became more rounded during treatment with serum-free medium and more flattened by bromodeoxyuridine (BrdU) treatment. In control cultures this subclone maintained the morphology of the parent cell line, but contained very low activity of choline acetyltransferase (CAT). RNA diversities of 6.5%, 6.8% and 8.5% of the uDNA and CAT specific activities of 0.64, 0.50 and 1.20 pmoles/min/μg DNA were found for control, no serum, and BrdU treated cells, resp. Treatment with dibutyryl-cyclic AMP failed to change the specific activity of CAT. A change to more fibroblastoid morphology, induced by BrdU, was associated with a marked increase in transcriptional diversity and a near doubling of CAT specific activity. These effects may reflect a change in the affinity of BrdU-substituted DNA for regulatory macromolecules. These data emphasize the difficulties encountered in attempts to correlate morphological and biochemical measures of differentiation.

888 EFFECT OF ACUTE AND CHRONIC ETHANOL ADMINISTRATION ON SYNAPTOSOMAL CALCIUM TRANSPORT. R. N. Seaman* and A. Y. Sun. Sinclair Comparative Medicine Research Farm, University of Missouri, Columbia, Missouri 65201

We have demonstrated that ethanol at low concentrations may affect some of the membrane-dependent transport processes in brain. In the present study, the effect of acute and chronic ethanol ingestion on Ca^{2+}-transport process in brain was investigated. Six-month-old Sprague Dawley male rats were used for this study and ethanol was administered by an intragastric intubation technique using a special ball-point needle. For acute administration of ethanol, three groups of rats were each given a single dose of saline or 30% (w/v) ethanol solution in the amount of 3 or 6 gm/kg. The Ca^{2+}-transport activity in brain synaptosomes was enhanced at the low ethanol level but was inhibited at the high dose level. Ethanol was also administered chronically by the intragastric tubing technique to another group of rats. In this instance, there was an increase in the Ca^{2+}-uptake activity of brain synaptosomes after prolonged alcohol ingestion. Results further indicated an increase of approximately 40% in Ca^{2+}-uptake activity in the chronic alcoholic group shortly after withdrawing the ethanol administration. These changes in synaptosomal Ca^{2+}-transport observed after acute and chronic ethanol administration may be related to the compartmentation of Ca^{2+} among subcellular membranes in brain. Alternately, a compensatory process counteracting the stress caused by ethanol may have occurred giving rise to the results obtained. (Supported by USPHS R01 AA02054-02).

889 EFFECTS OF D-LYSERGIC ACID DIETHYLAMIDE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT. Mami Shinohara*, Osamu Sakurada*, Jane Jehle*, and Louis Sokoloff. National Institute of Mental Health, Bethesda, Maryland, 20014, USA.

D-Lysergic acid diethylamide (LSD) is a potent psychotomimetic drug which produces profound disturbances in cerebral functions and perceptual aberrations including hallucinations. Although it is known to have effects antagonistic and agonistic to 5-hydroxytryptamine, the locus and mechanism of its psychotogenic effect are still unknown. The recently developed autoradiographic [^{14}C]deoxyglucose method provides a means to measure the rates of glucose consumption simultaneously in all the structural and functional components of the brain visible macroscopically in the autoradiographs. Because functional and metabolic activities are closely related, it also can be used to detect local alterations in cerebral functional activity. The method has been applied to the effects of LSD in the rat in an effort to define the areas of the brain with altered functional and metabolic activities. Over a dose-range from 12.5 to 125 μg/Kg LSD caused dose-dependent reductions in glucose utilization in selected cerebral structures. With increasing doses more and more structures were affected, and the effects were of greater magnitude. A pattern of distribution of effects among the various cerebral structures that might explain the drug's psychotogenic effects has thus far not been discernible.

890 MAMMALIAN CNS AXOLEMMA: HISTOCHEMICAL EVIDENCE FOR NEURONAL ORIGIN OF "AXOLEMMA-ENRICHED" MEMBRANE FRACTIONS. J. Stanley*, R. G. Saul*, M. G. Hadfield, and G. H. De Vries. Depts. of Biochemistry and Neuropathology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298 U.S.A.

Purified myelinated axons were isolated from fresh bovine brain by our method (Science 175, 1370, 1972) and osmotically shocked in EGTA to strip myelin and axolemma (AXL) from axon filaments. Four fractions were obtained from discontinuous sucrose gradient centrifugation: 1) a floating layer of myelin on 0.8 M sucrose, 2) & 3) trilaminar membrane vesicles and membrane fragments at the 0.8 M/1.0 M and 1.0 M/1.2 M sucrose interfaces (AXL) and 4) a pellet of "myelin-free axons". Electron microscopy (EM) of AXL revealed 50-800 nm trilaminar membrane vesicles. Multi-lamellar membrane vesicles characteristic of myelin were seldom seen. Light microscopy of histochemically stained fractions revealed many acetylcholinesterase (AChE) positive 300-1000 nm vesicles in the AXL. Staining was specific and was blocked by 5×10^{-5} M BW 284c51, 10^{-5} eserine, and 10^{-6} neostigmine bromide, but not by 8×10^{-5} tetraisopropylpyrophosphoramide. The myelin-free axons stained heavily AChE-positive though EM reveals relatively little associated membrane. Luxol Fast Blue, (LFB) staining was never seen in the myelin-free axon fraction but was positive for myelin, myelinated axon and AXL. The significant LFB staining of AXL was incommensurate with the small amount of microscopically discernible myelin contamination. The mammalian CNS axolemma could contain some constituent in common with myelin which is responsible for LFB staining. EM histochemical staining for AChE appeared to confirm light microscopic results. Based on histochemical reactivity we conclude that AXL is axonal plasma membrane in origin. (Supported by NIH Grant NS10821-03)

891 THE EFFECTS OF CARBAMYLCHOLINE CHLORIDE ON ARACHIDONATE METABOLISM IN THE MOUSE BRAIN. Kwei L. Su* and Grace Y. Sun. Sinclair Comparative Medicine Research Farm, Univ. of Missouri, Columbia, MO 65201.

The stimulation of ^{32}Pi incorporation into the phosphatidic acids (PA) in rodent brain by carbamylcholine (CC) has been demonstrated in vivo and in vitro. However, the effect of CC on brain fatty acid metabolism has not been investigated. A control group of mice was injected intracerebrally with [1-^{14}C]-arachidonate and an experimental group was injected with the same precursor plus CC (4 µg/brain). At 1, 3 and 10 min after injections, brains were removed and homogenized. Aliquots of total brain homogenates were also fractionated into microsomes and synaptosome-rich fractions by sucrose gradient centrifugation. All three fractions were analyzed for the incorporation of radioactivity from [1-^{14}C]-arachidonate into various lipid classes. In general, the turnover of labeled arachidonate in the brain injected with CC was slower which corresponded to a decreased incorporation of radioactivity into the choline and ethanolamine phosphoglycerides. However, an increase in radioactivity was found among the PA, diacylglycerols (DG) and triacylglycerols (TG) in the CC group. The increase in DG and TG labeling was more obvious in the synaptosome-rich fraction than in the microsomal fraction. Results are in agreement with previous in vitro findings by Schacht and Agranoff (J.B.C. 249:1551, 1974) demonstrating the stimulation of phosphatidate phosphohydrolase by CC in brain synaptosomal preparations. With arachidonate as labeled precursor, this event leading to increased DG and TG labeling in the synaptosomes can be further illustrated in the in vivo system. (Supported by USPH NS 12960).

892 ACUTE AND CHRONIC EFFECTS OF MORPHINE ON GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY AND γ-AMINOBUTYRIC ACID (GABA) LEVEL IN DISCRETE BRAIN AREAS OF THE MOUSE. A. Takanaka*, S. F. Tzeng* and I. K. Ho. Dept. of Pharmacol. and Toxicol., Univ. of Miss. Med. Ctr., Jackson, MS 39216, U.S.A.

Thirty min after male ICR mice received morphine sulfate (30 mg/kg, s.c.), GAD activity in whole brain remained the same as that of the control group receiving saline. However, there was an increase of 11% in brain level of GABA. GAD activity was elevated 12% but GABA level remained unchanged in cerebral cortex. In cerebellum, GAD activity remained the same but GABA level had a 20% decrease. No change in GAD activity or GABA level was detected in brain stem, hypothalamus and the remaining area of the brain. On the other hand, in animals rendered tolerant-dependent by implanting a specially formulated 75 mg morphine pellet for 72 hr, the treatment conveys about a ten-fold tolerance to the antinociceptive response to morphine. Brain GAD activity and GABA level were decreased 35 and 10%, respectively. GAD activity in cerebellum and hypothalamus of morphine pellet implanted mouse was 13 and 42% lower. GAD activity in cerebral cortex, brain stem and the remaining area remained the same. GABA levels in cerebral cortex and hypothalamus were 19 and 22% lower, respectively. No change was found in cerebellum and the remaining area of the brain. However, GABA level was elevated 15% in the brain stem area. After abrupt withdrawal from morphine for 7 and 24 hr, brain GAD activity was further decreased to 50 and 87%, respectively, to that of the placebo control group. The brain GABA levels at those time intervals still remained significantly lower. These data further support our previous finding that GABA system is involved in morphine analgesia, tolerance and physical dependence. (Supported by NIDA grant DA-01310 and General Research Support Grant RR05386.)

893 SEROTONIN BINDING PROTEIN (SBP): MECHANISM OF ENHANCEMENT OF BINDING BY Fe^{+2}. Hadassah Tamir, Jack Peisach,* and Maurice M. Rapport. N.Y. State Psychiatric Institute and Albert Einstein College of Medicine.

In previous publications (Tamir et al, 1974, 1976) we reported the presence of a soluble protein, found in both central and peripheral nervous systems, with high affinity for serotonin. This protein is found in serotonergic tracts and binds newly synthesized serotonin. Fe^{+2} (but not Fe^{+3}) enhances serotonin binding many fold after the Fe^{+2} is bound to the protein. We now find that the mechanism of enhancement does not involve oxidation of Fe^{+2} to Fe^{+3} either before or after serotonin binding as judged from an absence of EPR signal near g=4.3. In contrast, aerobic addition of other protein (albumin) to Fe^{+2} causes rapid oxidation to Fe^{+3}. In the presence of phosphate, Fe^{+2} induces aggregation of SBP, as determined by centrifugation on a glycerol gradient. This aggregation suggests the formation of -S-Fe-S- bonds since both PCMB (thiol group blocker) and DTT (thiol reducing agent) inhibit serotonin binding to the SBP-Fe^{+2} complex. Free radical intermediates that are oxygen mediated are ruled out by the fact that DTT inhibits serotonin binding anaerobically. It is concluded that Fe^{+2} enhances binding of serotonin to SBP by causing an aggregation of the protein, probably via sulfhydryl groups.

894 RADIOCHROMATOGRAPHIC SCREENING FOR NEUROTRANSMITTERS IN LIMULUS.
James G. Townsel, Henry E. Baker*, and Thyckla T. Gray*, Department of Physiology, Meharry Medical College, Nashville, Tennessee 37208.

The neurogenic heart of Limulus receives cardioregulatory inputs from the brain as well as the chain of abdominal ganglia {Pax, R.A. Comp. Biochem. Physiol. 28:293 ('69); Von Burg, R. and Corning, W.C. Can. J. Physiol. Pharmacol. 48:333 ('70)}. Acetylcholine (ACh) has been suggested as the cardioexcitatory transmitter, and both γ- aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT, serotonin) are regarded as candidates for the role of cardioinhibitory transmitter. Preliminary transmitter screening of each of the abdominal ganglia has been accomplished. Our screening experiments consist of short term sterile cultures of abdominal ganglia in an appropriate medium containing the radiolabelled transmitter precursors, choline, glutamate, tyrosine, and tryptophan. All of these precursors were taken up in significant proportions by the tissues. None of the expected transmitter candidates arise from ^{14}C- tyrosine or ^{14}C- glutamate. Of the ^{14}C- choline taken up, from 10 to 25 percent is incorporated into ACh. We observed an inconsistent appearance of label in the region co-electrophoresing with 5-HT in extracts of tissue incubated with ^{14}C- tryptophan. The use of media containing DL-5-hydroxy (methylene -^{14}C-5-HTP) resulted in poor uptake of this compound (less than five percent). However, between 25 and 45 percent of the DL-^{14}C-5-HTP taken up was converted to a metabolite which co-electrophoresed with 5-HT.

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895 CHARACTERISTICS OF THE VERATRIDINE-INDUCED RELEASE OF AMINOACIDS FROM PINCHED OFF NERVE ENDINGS.
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We have studied the effects of veratridine on the release of radiolabelled GABA, Proline and Aspartate from pinched off nerve endings isolated from the rat brain. The drug produces a sodium dependent, tetrodotoxin-sensitive depolarization of the nerve membrane (Physiol. Rev. 54: 813, 1974). Veratridine (2×10^{-4}M) induces a five-fold increase in the rate of release of all these aminoacids. The increase in the rate of release was abolished by replacing sodium by choline in the outside solution or by the addition of tetrodotoxin (TTX 5×10^{-5}M). These findings show that the veratridine induced release of aminoacids is mediated by a depolarization with a mechanism homologous to that induced by the nerve action potential. In other experiments we examined the Ca++ dependence of the veratridine induced release. Lack of Ca++ or increasing outside Mg++ or Mn++(15mM) did not block veratridine-induced release of GABA. These findings suggest the possibility that in the nerve terminals, aminoacids can be released by a mechanism that is triggered by depolarization but is not Ca dependent. Another possibility is that elements other then nerve terminals - perhaps of neuroglial origin - could have TTX sensitive Na channels activated by veratridine; this activation would trigger aminoacid release.

896 DIFFERENCES IN THE REGULATION OF ACETYLCHOLINE TISSUE LEVELS AND ACETYLCHOLINE RELEASE IN DIFFERENT REGIONS OF RAT BRAIN AND THE EFFECTS OF PENTOBARBITAL. Marshall B. Waller* and Judith A. Richter (Spon. J. Nurnberger), Inst. of Psychiatric Research and Dept. of Pharmacology, Indiana University School of Medicine, Indianapolis, IN. 46202.

Pentobarbital (PB), when administered to rats in vivo, increase brain levels of acetylcholine (ACh). It has been suggested that the ACh content increases as a result of the inhibition of ACh release and a subsequent accumulation of the transmitter. Since this proposal has not been tested directly for the barbiturates, we compared the effect of PB on the release of ACh and the tissue levels of ACh from different regions of rat brain. Sodium pentobarbital (50 mg/kg, i.p.) increased ACh levels in the cerebral cortex, striatum, hippocampus and pons-medulla but not in the midbrain; however, PB (5×10^{-4}M) inhibited potassium-stimulated ACh release from all regions in vitro. The release of ACh and tissue levels of this transmitter were compared directly during in vitro superfusion (in the presence of paraoxon) of prisms prepared from the cerebral cortex and midbrain. Stimulation by 50 mM KCl released a similar amount of ACh from both regions: 36.3 nmoles/g/90 min from cortex and 32.4 nmoles/g/90 min from midbrain. This release was accompanied by a 56% decrease in the cortical ACh content but there was no significant change in ACh in the midbrain. PB inhibited the stimulated ACh release from both regions. Treatment of cortex with PB during stimulation with 25 mM KCl increased cortical ACh content back to the unstimulated level. However, in the midbrain, PB inhibition of ACh release stimulated by 25 or 50 mM KCl did not concomitantly increase tissue ACh levels. These results suggest that the regulation of ACh synthesis and release is different in the two brain regions and that PB can inhibit ACh release without necessarily causing a concomitant rise in tissue ACh content. (Supported by Grant No. DA 60796-02).

897 PHOSPHOPROTEIN PHOSPHATASE: EVIDENCE FOR ACTIVE AND INACTIVE FORMS OF THE ENZYME. H.-Y.T. Yang,* E. Costa, E.A. Majane* and J. Hong* Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hosp., Washington, D.C. 20032

Both soluble and particulate fractions of rat pineal catalyze the dephosphorylation of phosphohistone. Further fractionation of the soluble fraction of pineal homogenates by Sephadex G-200 column revealed the existence of two forms of phosphoprotein phosphatase differing in molecular weight. Addition of deoxycholate to a crude soluble fraction resulted in three fold activation of the enzyme. Furthermore, it was shown that the high molecular weight form but not the low molecular weight form was activated by deoxycholate. Fractionation of the deoxycholate activated enzyme showed that activation of the phosphatase was due to an increase of the low molecular weight form of the enzyme. The phosphatase in cytosol as well as in particulate was inhibited by $ZnCl_2$ and NaF. Guanosine triphosphate and ATP was found to activate the soluble enzyme but not the enzyme in particulate fraction. A thermostable factor which activates the crude but not the deoxycholate stimulated enzyme was demonstrated in both beef and rat pineal glands. The result provided evidence for the existence of an active and inactive form of phosphoprotein phosphatase in pineal gland. Whether the endogenous activator mediates the regulation of the phosphoprotein phosphatase in pineal gland remains to be investigated.

898 THE NEUROFILAMENT PROTEIN IS A MAJOR COMPONENT OF THE POST-SYNAPTIC DENSITY. S-H. Yen*, Paul Kelly, Ronald Liem*, Carl Cotman and Michael L. Shelanski* (SPON: M.M. LaVail). Dept.Neuropath. Harvard Medical School, Boston, MA. 02115 and Dept. Psychobiol. U.Calif.Irvine, Irvine, CA.

Antiserum prepared against bovine brain 10nm filaments in the rabbit by the method of Yen et al. (PNAS 73:529,1976) was used to localize the filament antigen at the electronmicroscopic level using indirect immunoperoxidase methods in the mouse cerebrum. In addition to the expected localization to the filaments of neurons and glia, prominent staining was seen in the post-synaptic thickening. Neither filaments or thickenings were stained when preimmune rabbit serum was used. The presence of the filament antigen in the post-synaptic density (PSD) was further supported by studies on purified PSDs. Densities isolated from rat brain (Banker et al., J.Cell Biol. 63:441,1974) gave a reaction of identity with bovine brain filaments when tested by immunodiffusion against the anti-filament antiserum. The anti-filament antiserum used in these studies does not react against native or denatured tubulin, nor against either of the tubulin subunits, on immunodiffusion or on radioimmunoassay. The purified densities show a number of bands in the 50 - 60,000 dalton region with the major band at 52,000. This major band has the same molecular weight as the filament protein and both migrate slightly ahead of the β-tubulin band on discontinuous SDS gel electrophoresis. Walters and Matus (Nature, 257:498,1975) have reported staining of the PSD in tissue with an anti-tubulin antibody and strong similarity in the peptide maps of tubulin and the major protein of the PSD. However, peptide map similarities exist between α-tubulin and filament protein as well, while the filament molecular weight is lower than that of tubulin and the same as the filament. These results do not argue against the presence of tubulin at the density, but do suggest the filament protein is the major component.